VOLUME 8 • NUMBER 5 • March 1956

JOURNAL OF

Applied Physiology

Contents

Published by

THE AMERICAN PHYSIOLOGICAL SOCIETY

Journal of APPLIED PHYSIOLOGY

The Journal of Applied Physiology is published bi-monthly, in one volume per year, by the American Physiological Society, Inc. *Subscription price:* $7.50 per year postpaid in the United States and countries of the Pan American Postal Union. Postage to Canada, 50 cents per year extra; postage to other countries, $1.00 per year extra. Single copies, $2.00 each postpaid. Subscriptions from outside the United States must be payable in United States currency or full equivalent.

Entered as second class matter September 10, 1948, at the Post Office, Washington 13, D. C., under the Act of March 3, 1879. Accepted for mailing at special rate provided for by Section 538, P. L. & R., Paragraph 4, Act of February 28, 1925. Printed at the Waverly Press, Inc., Baltimore, Maryland.

Address all business correspondence to:

JOURNAL OF APPLIED PHYSIOLOGY

9650 Wisconsin Avenue Washington 14, D. C.

JOURNAL OF APPLIED PHYSIOLOGY March 1956

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Journal of
APPLIED
PHYSIOLOGY

VOLUME 8 *March 1956* NUMBER 5

Beat-to-Beat Alterations in Relationship of Simultaneously Recorded Central and Peripheral Arterial Pressure Pulses During Valsalva Maneuver and Prolonged Expiration in Man[1]

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IN A COMPARISON of simultaneously recorded central and peripheral arterial pressure pulses in the steady state in varying physiologic conditions, certain changes that the pulse undergoes in its transmission peripherally have become manifest (1). In order to study the constancy of these relationships and in the hope of gaining some insight into the mechanisms responsible for the striking alterations in contour as the pressure pulse is transmitted peripherally, central and peripheral pressure pulses at multiple sites were recorded simultaneously during the rapid alterations in the cardiovascular status of normal persons induced by the Valsalva maneuver or a prolonged expiration.

The Valsalva maneuver has been well documented (2). Supposed to have been used by the ancients to commit suicide (3), it was used by Valsalva and his pupil, Morgagni (4), to study the tympanic membrane in the living subject by expiratory pressure against a closed outlet in the region of the mouth and nose and also in the form of expiratory pressure against a closed glottis so that "such pressure can be exerted upon the heart and intrathoracic vessels that the movement and flow of blood are temporarily arrested" (5).

The circulatory changes occurring during the Valsalva maneuver have also been described more recently (6, 7). Our studies were not designed to supplement these but rather to investigate the beat-to-beat changes in the relationship of the pressures and contours of the central and peripheral arterial pressure pulses generated by the same heart beats under these conditions of transitory cardiovascular stress.

METHOD

The studies consisted of numerous Valsalva maneuvers done by five healthy physicians whose average age was 35 years (range: 31–45), average height was 69 inches (range: 66–71) and average weight was 169 pounds (range: 145–205). All Valsalva maneuvers were done with the subjects in a 40-degree head-up tilt position and all pressures were referred to the midchest at the level of the sternal end of the third interspace. After a full inspiration, an expiratory pressure of 40 mm Hg was transmitted by the subject through an open glottis via a mouthpiece to a Tycos manometer used for visual monitoring and voluntary control of the pressure level. A side tube from the Tycos tubing was connected to a calibrated strain gauge to produce a graphic tracing on the photokymographic record. Duration of the Valsalva maneuvers varied from 10 to 22 seconds.

Central pressure pulses were recorded through a Peterson-type arterial catheter advanced up to the arch of the aorta or to the left subclavian artery through an 18T-gauge needle inserted into the femoral

Received for publication June 13, 1955.

[1] Abridgment of portion of thesis submitted by Dr. Kroeker to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine.

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artery at the groin or into the left brachial artery in the antecubital fossa, respectively. The final position of the catheter tip as measured on the roentgenogram with correction for x-ray tube distortion was an average of 7 cm (range: 4.2–9.3) from the top of the aortic arch at the mid-sternal line. The strain-gauge catheter system had a frequency response which was uniform out to 12 cycles/sec. Peripheral arterial pressure pulses were recorded by means of a hypodermic strain-gauge manometer system as previously described (8, 9) with a uniform response to sine wave pressure variations up to 25 cycles/sec. Dynamic response characteristics of all manometer systems to sine and square pressure variations were determined immediately following each procedure and were considered to be adequate for recording of all practically important components of the central and peripheral arterial pressure pulses (10, 11).

Photokymographic recording was at paper speeds ranging from 75 to 150 mm/sec. Pressure calibrations were done frequently during the course of the procedure, known pressures being projected into all the manometer systems simultaneously. Mean pressures were obtained by planimetry of the photographic record. Central and radial pressure pulses of all subjects were simultaneously recorded, three subjects had brachial and radial needles in the same arm, and one subject had in addition femoral and dorsalis pedis intra-arterial needles. In the latter subject, prolonged expiration to the limit of endurance with the glottis open was done in the supine position.

RESULTS

Figure 1 illustrates the striking beat-to-beat changes in central and peripheral arterial pulse contours and their interrelationships produced by the Valsalva maneuver and by a prolonged expiration. The upper panel at the left shows control pulses just before a 20-second Valsalva maneuver with mouthpiece pressure of 40 mm Hg and subject at the 40-degree head-up tilt. On the right are the pulses recorded at the end of the Valsalva maneuver and beginning of normal breathing. The lower panel at the left shows control pulses in the same subject in the supine position. On the right are the pulses recorded during prolonged expiration. This panel shows the right radial, left femoral and left subclavian pulses as in the upper panel, but in addition pulmonary trunk pressures were recorded via a Cournand-type cardiac catheter (F. 6) threaded through the right heart. An

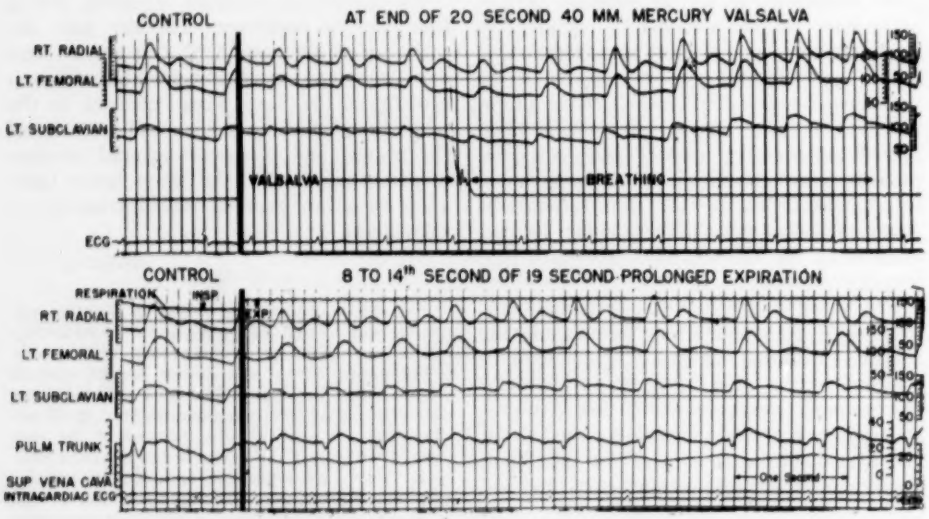

FIG. 1. Rapid changes in arterial pulse contours produced by respiratory maneuvers. Photokymographic recording at a paper speed of 150 mm/sec. of effect of Valsalva maneuver and of a prolonged expiration on pressure pulses of a 31-yr.-old normal man (*subject 2*). Vertical lines represent 100-msec. intervals. *Upper:* At *left* are control pulses of right radial and left femoral arteries, and of left subclavian artery near the aorta, and electrocardiogram recorded at the 40-degree head-up tilt just prior to a 40 mm Hg pressure Valsalva maneuver. At *right* are pulses recorded toward the end and immediately following the 20-sec. Valsalva maneuver. The end of Valsalva maneuver is indicated by a return to baseline pressure of the strain-gauge trace recording the intraoral pressure. *Lower:* At *left* are control pulses of same subject in supine position. This record shows, in addition, simultaneous recording of pulmonary arterial and superior vena caval pressures. At *right* are pulses recorded during the 8th–14th sec. of a 19-sec. period of forced expiration. See text for discussion of changes in contours of pressure pulses.

electrode recorded the electrocardiogram from the tip of this catheter. Another Cournand-catheter recorded pressure from the superior vena cava.

The control pulses in the upper panel show the effects of the 40-degree head-up tilt. There is an increase in heart rate, shortening of the duration of systole and a decrease in the prominence of the secondary wave in the radial pulse as compared with the control pulses in the lower panel taken somewhat earlier in the course of the same procedure with the subject supine. There is also a decrease in the central (subclavian) pulse pressure during the tilt but an increased amplification of this pulse pressure in the femoral and radial arteries.

The upper panel at the right shows the same pulses at the end of the 20-second Valsalva maneuver. The changes in the pulse initiated by the 40-degree tilt have been magnified. The heart rate has increased from 80 beats/min. in the control pulses on the left, to 100 beats/min. at the end of the Valsalva maneuver. The duration of systole is markedly shortened and there is complete disappearance of the secondary wave in the radial pulse with the development of a deep dicrotic notch, lower than end-diastolic pressure so that the radial pulse resembles a damped sine wave. The central pulse (subclavian) shows a great reduction in pulse pressure, disappearance of a discernible anacrotic wave and a marked shortening of build-up time. Radial pulse pressure amplification (radial pulse pressure/subclavian pulse pressure) increased, radial pulse pressure being two and a half times the central pulse pressure. Femoral pulse pressure amplification was decreased. The onset of the femoral pulse precedes the onset of the radial pulse in the control, while during the Valsalva maneuver the onset of the radial pulse precedes the onset of the femoral pulse. After the Valsalva maneuver the pulse rapidly returns to a more normal contour. There is a lengthening of the duration of systole despite a maintenance of a rapid heart rate in the 4-second period immediately following the Valsalva maneuver. With the lengthening of systolic time there is a gradual reappearance of the secondary wave in the radial pulse.

The lower panel, recorded during prolonged expiration, shows similar contour changes in the systemic arterial pulses. Heart rate increased from 75 beats/min. in the control to 120 beats/min. after 9 seconds of prolonged expiration. The radial pulse contour was similar to that seen during the Valsalva maneuver, and radial pulse pressure was increased in relation to the subclavian to a maximal level of 290%. There was some increase in femoral pulse transmission time (decrease in pulse wave velocity). Also there was an increase in femoral pulse pressure amplification relative to the subclavian, whereas there was a decrease of femoral pulse amplification during the Valsalva maneuver, as already mentioned. In the

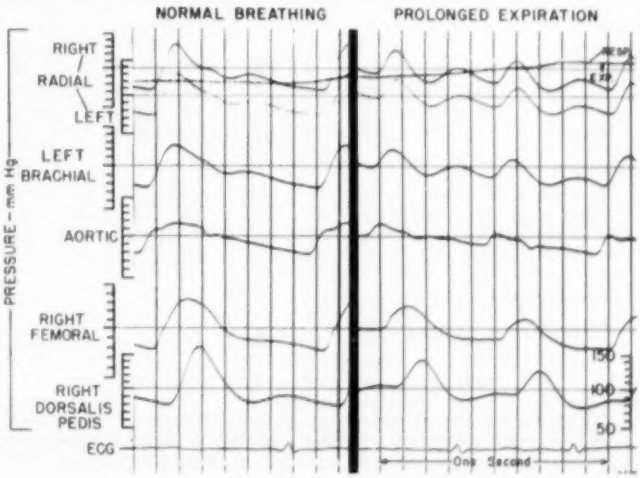

FIG. 2. Comparison of central and peripheral arterial pulses (normal subject during prolonged expiration). Photokymographic recording of multiple arterial pressure pulses at a paper speed of 150 mm/sec. At *left* are pressure pulses in supine position during normal breathing. At *right* are pressure pulses recorded during maximal pressure pulse effects produced by a prolonged expiration.

first three pulses of the prolonged expiration record, the onset of the femoral pulse is superimposed on the ascending pressure following the dicrotic dip of the previous pulse. These three pulses show the peak of femoral pulse pressure amplification. After the fourth pulse this phase relationship is altered and the amplification relative to the subclavian pulse pressure immediately drops by 30%.

The pressures in the main pulmonary artery and superior vena cava are both increased during the prolonged expiration. The pressure pulse recorded from the pulmonary artery is distorted by artifact probably due to motion of the catheter (12).

Figure 2 further illustrates the changes in the pressure pulses during prolonged expiration in simultaneously recorded right and left radial, left brachial, aortic, right femoral and right dorsalis pedis pressure pulses. The recording on the right shows more clearly the increase in femoral pulse transmission time during the prolonged expiration. (The complete pressure and time relation analyses of the pressure pulses from the procedure from which this illustration was taken are given in tables 4 and 5.)

Table 1 shows the effect of the Valsalva maneuver and 40-degree head-up tilt on the relationship between pressures in the aorta and in the brachial and radial arteries. The control values represent three consecutive pulses taken immediately prior to the deep inspiration preparatory to the Valsalva maneuver. The Valsalva values represent three consecutive pulses taken at the point where the greatest change in contour (dicrotism) occurred, 5–8 seconds after the onset of the Valsalva maneuver.

Aortic systolic pressure decreased in all subjects an average of 16 mm Hg, while the effect on diastolic pressures was not uniform, the average effect being an increase of 3 mm during the Valsalva maneuver. The pulse pressure in the aorta was reduced in each subject, the average reduction being 19 mm. The dicrotic pressure was uniformly decreased, the average reduction being 16 mm, and on occasion was lower than end-diastolic pressure. Mean pressure was reduced an average of 8 mm.

Simultaneous peripheral brachial and radial pressures in the same arm (right) showed the same trends of pressure change during the Valsalva maneuver, although mean pressures dropped somewhat less than in the central

TABLE 1. EFFECT OF VALSALVA MANEUVER ON RELATIONSHIP BETWEEN PRESSURES* GENERATED BY SAME HEART BEATS IN AORTA, BRACHIAL AND RADIAL ARTERIES†

Subj.	Aortic					Brachial					Radial				
	Systolic	Diastolic	Dicrotic	Pulse	Mean	Systolic	Diastolic	Dicrotic	Pulse	Mean	Systolic	Diastolic	Dicrotic	Pulse	Mean
Control															
3	115	80	101	35	97	137	77	92	60	97	147	78	90	69	97
1	112	78	101	34	94	128	77	98	51	93	132	77	95	55	94
4	124	78	105	46	100	131	73	97	58	95	132	72	93	60	95
Av.	117	79	102	38	97	132	76	96	56	95	137	76	93	61	95
Valsalva‡															
3	105	80	90	25	91	122	79	77	45	90	130	81	73	57	90
1	94	75	74	20	81	100	74	70	30	81	104	75	67	37	82
4	103	89	95	14	96	111	86	86	25	96	111	85	84	27	93
Av.	101	81	86	20	89	111	80	78	33	89	115	80	75	40	88
Decrease during Valsalva															
3	10	0	11	10	6	15	−2	15	15	7	17	−3	17	12	7
1	18	3	27	14	13	28	3	28	21	12	28	2	28	18	12
4	21	−11	10	32	4	20	−13	11	33	−1	21	−13	9	33	2
Av.	16	−3	16	19	8	21	−4	18	23	6	22	−5	18	21	7

* Pressures expressed in mm Hg. † Simultaneous recordings in 3 normal subjects with radial and brachial needles in same arm arranged in order of magnitude of change in pulse pressure produced by Valsalva maneuver. ‡ Measurement of 3 consecutive pulses at peak of central pulse pressure amplification peripherally which occurred at the 5th–8th sec. of Valsalva maneuver.

pulse so that central and peripheral mean pressures were practically identical. Brachial and radial dicrotic pressures were uniformly lower than end-diastolic pressures, an average of 2 and 5 mm Hg, respectively, so that peripheral pulse pressure now consisted of the pressure difference between systolic and dicrotic pressures.

Table 2 shows the effect of the Valsalva maneuver and 40-degree head-up tilt on the relationship between aortic and radial arterial pulse pressures in five normal men. The average decrease in central pulse pressure during the Valsalva maneuver from the control values was 21 mm Hg. However, radial pulse pressure was 165% of aortic at the 40-degree tilt and rose to 207% at the peak effect of the Valsalva maneuver.

TABLE 2. EFFECT OF VALSALVA MANEUVER AT 40-DEGREE TILT ON RELATIONSHIP BETWEEN AORTIC AND RADIAL ARTERIAL PULSE PRESSURES*

Condition	Constant	Aortic Pulse Pressure, mm Hg	(Radial Pulse Pressure/Aortic) × 100, %
Control	Average	41	165
	Range	34–50	122–194
Valsalva†	Average	20	207
	Range	14–24	180–233
Decrease during Valsalva	Average	21	−42
	Range	11–36	−15 to −78

* Average values: 5 normal men.

† Average of 3 consecutive pulses at peak of peripheral amplification of pulse pressure which occurred at 5th–10th sec. of Valsalva maneuver.

Table 3 shows the effect of the Valsalva maneuver and the 40-degree head-up tilt on the time relations of simultaneous pressure pulses in the aorta and brachial and radial arteries. The time from the peak of the R-wave to the onset of the aortic pulse, already prolonged by the tilt (113 msec.), was further prolonged an average of 19 msec. during the Valsalva maneuver. Aortic build-up time was shortened by 102 msec., while systolic time was shortened by 53 msec. Brachial and radial build-up times were similar in the control (100 msec.) and were shortened 18 and 14 msec., respectively, so that the radial build-up time at the peak of pulse pressure amplification during the Valsalva maneuver was actually somewhat longer than the build-up time of the simultaneous brachial pulse. There was little change in the transmission time from the onset of the aortic pulse to the onset of the pulse wave at the

TABLE 3. EFFECT OF VALSALVA MANEUVER ON TIME RELATIONS* OF SIMULTANEOUS PRESSURE PULSES IN AORTA AND BRACHIAL AND RADIAL ARTERIES†

Subj.	Heart Rate, Beats/min.	Aortic			Brachial		Radial	
		R-wave to onset	Build-up (onset to peak)	Systolic	Transmission time‡ (aortic to onset)	Build-up (onset to peak)	Transmission time (aortic to onset)	Build-up (onset to peak)
Control§								
3	78	98	163	266	47	92	62	97
1	69	125	107	260	47	100	68	97
4	72	116	195	272	46	108	63	107
Av.	73	113	155	266	47	100	64	100
Valsalva§								
3	81	108	59	235	52	86	64	87
1	84	156	46	204	50	75	73	79
4	91	133	55	200	46	86	63	93
Av.	85	132	53	213	49	82	67	86
Decrease during Valsalva								
3	−3	−10	104	31	−5	6	−2	10
1	−15	−31	61	56	−3	25	−5	18
4	−19	−17	140	72	0	22	0	14
Av.	−12	−19	102	53	−3	18	−2	14

* Time intervals expressed in milliseconds. † Data from 3 normal subjects with brachial and radial needles in same arm. ‡ Transmission times were measured from onset of aortic pulse to onset of peripheral pulse. § Each figure is an average value for 3 consecutive pulses taken during control period and 5th–8th sec. of Valsalva maneuver.

brachial and radial arteries. In the control pulses the radial systolic peaks on the average occurred 17 msec. later than the brachial peaks. During the peak amplification of these pulses during the Valsalva maneuver, this figure was increased to 22 msec. Heart rates were increased an average of 12 beats/min. in the Valsalva maneuver as compared with the control tilt values.

Tables 4 and 5 show the effect of a prolonged expiration on the pressure and time relations of simultaneous pressure pulses from the aorta and multiple peripheral arteries, with the subject supine, from the procedure illustrated in figure 2. Note the increase in amplification of the aortic pulse pressure peripherally, maximal values being obtained in the dorsalis pedis artery, the shortening of the duration of systole during this period of peak peripheral amplification of the pulse pressure and the increase of heart rate.

TABLE 4. EFFECT OF PROLONGED EXPIRATION ON RELATIONSHIP BETWEEN SIMULTANEOUS PRESSURES* GENERATED BY SAME HEART BEAT IN AORTA AND MULTIPLE PERIPHERAL ARTERIES (SUBJECT 2)

Condition (heart rate)	Site	Pressure, mm Hg					Pulse Press. Ratio: Peripheral / Aortic
		Systol.	Diastol.	Dicrot.	Mean	Pulse	
Control (81)	R. radial	138	77	92	98	61	1.51
	L. radial	136	77	92	97	59	1.46
	L. brach.	131	79	95	99	52	1.29
	Aorta	122	81	104	100	41	
	R. fem.	138	80	93	100	59	1.45
	R. dorsal. pedis	166	81	85	102	86	2.11
Peak amplification† (115)	R. radial	135	101	91	108	44	2.24
	L. radial	130	102	95	109	35	1.78
	L. brach.	128	104	99	109	29	1.49
	Aorta	125	106	105	112	20	
	R. fem.	136	105	101	114	33	1.78
	R. dorsal. pedis	153	110	98	115	55	2.81
Normal breathing‡ (72)	L. radial	173	96	118	121	77	1.44
	L. brach.	166	98	121	122	68	1.26
	Aorta	154	100	132	125	54	
	R. fem.	175	100	118	124	74	1.37
	R. dorsal. pedis	207	102	107	126	107	1.98

* Each value is average of 3 consecutive pulses.
† At 5th sec. of a prolonged expiration.
‡ 3 sec. after resumption of normal breathing.

TABLE 5. EFFECT OF PROLONGED EXPIRATION ON TIME RELATIONS OF SIMULTANEOUS PRESSURE PULSES* IN AORTA AND MULTIPLE PERIPHERAL ARTERIES (SUBJECT 2)

Condition	Site	R-wave to Onset	Transmission Time to Onset†	Build-up Time (Onset to Peak)	Systolic Time
Control	R. radial		59	102	340
	L. radial		65	103	350
	L. brach.		37	113	345
	Aorta	100		170	314
	R. fem.		61	151	363
	R. dorsal. pedis		131	127	360
Peak amplification	R. radial		44	82	224
	L. radial		46	84	233
	L. brach.		25	87	236
	Aorta	121		56	216
	R. fem.		67	109	
	R. dorsal. pedis		136	100	276
Normal breathing	L. radial		61	99	330
	L. brach.		36	110	330
	Aorta	84		179	303
	R. fem.		53	148	351
	R. dorsal. pedis		115	119	349

* See table 4 for pressure levels of these pulses.
† Transmission time measured from onset of aortic pulse to onset of peripheral pulse. All values in milliseconds.

COMMENT

In addition to the marked relative increase of the pulse pressure in peripheral arteries in relation to the central pulse observed during the Valsalva maneuver and during prolonged expiration, there was a disappearance of the secondary wave in the brachial and radial pulses and a dipping down of dicrotic pressures to below end-diastolic pressures so that the general appearance of the pulse was similar to that of a simple sine wave. This is the resonant peripheral pulse described by Frank (13). He

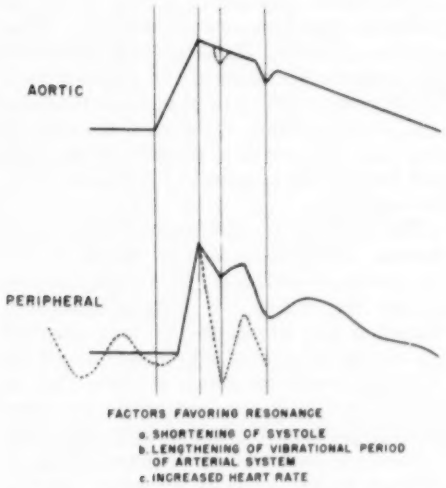

FIG. 3. Resonance phenomena in peripheral arterial pulse, as modified from Frank. With shortening of systole the incisura of aortic pulse (*dotted*) will be in phase with descending limb of primary peripheral pulse wave, causing a resonance phenomenon and a dipping down (*dotted line*) of that limb to below diastolic level. A similar phenomenon could theoretically result from lengthening of the vibrational period of peripheral arterial system (distance between primary and secondary peak) so that the incisura again would be in phase with descending limb of the primary pulse wave. A third factor that could produce resonance would result if vibrational energy were still present at the onset of systole (*dotted line* preceding onset of peripheral pulse). In this case positive ascending limb of central pulse would be in phase with a rising peripheral pressure. This would cause increase in primary peripheral systolic peak pressure. This condition would be favored by rapid heart rates and decreased damping in the arterial system.

observed it in high fevers and produced it in normal subjects by the administration of amyl nitrite. In all cases he noted a concomitant shortening of the time of systole.

Figure 3 presents a diagrammatic explanation of the possible genesis of this pulse, as modified from Frank. Assuming the absence of all vibrational energy at the onset of systole, the central pulse consists of two forcing impulses: a positive, the ascending limb; and a negative, smaller impulse as a small quantity of blood returns to the left ventricle before closure of the semilunar valves, the incisura. The distance between these two impulses is the duration of systole (not strictly so, since this measures the interval between the onset of one impulse and the peak of the next). If by shortening of systole (to point of dotted incisura in aortic pulse of diagram, fig. 3) the negative impulse occurred soon enough to be in phase with the descending portion of the primary peripheral pulse wave, the peripheral arterial system could be considered to be in resonance with the central pulse, resulting in great changes of contour (dotted primary and secondary peak in diagram of peripheral pulse) and amplification of pulse pressure.

The same effect could theoretically be produced by lengthening the vibrational period of the arterial system (systolic time remaining constant), and this factor may have played a part in the resonance produced by Frank with amyl nitrite, where, although there is a shortening of the time of systole, there is also a marked lowering of blood pressure.

A third and possibly minor factor not mentioned by Frank could arise if vibrational energy from the previous cardiac impulse were still present at the beginning of the next systole (dotted line preceding onset of peripheral pulse in diagram, fig. 3, and illustrated by femoral pulse in lower panel of fig. 1). The positive impulse of the systolic upstroke might then be in phase with a rising peripheral pressure and cause a resonance phenomenon, increasing the magnitude of the initial upswing. This could be suspected not only from the contour of the previous diastolic limb but from the fact that the initial amplitude of the upswing might be more than twice the aortic pulse pressure. An example of this phenomenon in the femoral pressure pulses of a normal subject is shown in figure 1 (first three pulses of left lower panel).

The peripheral arterial system during the Valsalva maneuver lends itself particularly well to the analogy of its acting as an inadequate, underdamped manometer system responding to an impulse (the central pulse) of a frequency in the range of its own resonant frequency. Thus in these maneuvers a rapid central build-up of the pressure pulse is transmitted down the system with progressive prolongation of the build-up time and increase in amplitude.

The complicating factors of possible inconstancy of the system (type of central pressure

pulse (14), effective length of arterial segment (15), damping (16), peripheral resistance (17), being kept in mind, the duration of systole was plotted against peripheral pulse amplification of the central pulse as in the classic frequency response curve of a manometer system.

In figure 4 the duration of systole (onset of central pulse to incisura) in milliseconds during the Valsalva maneuver is plotted on the abscissa and peripheral-aortic pulse pressure ratios on the ordinate. *Subject 2* (top panel) started at a high level of 160% radial amplification of central pulse pressure at 260 msec. duration of systole, and this progressively increased with shortening of systole in the Valsalva maneuver to a maximum of 235% amplification of central pulse pressure at 200 msec. duration of systole. Simultaneous femoral pulses showed less correlation of amplification to the duration of systole, although amplification seemed to decrease with the shortening of systole. *Subject 1* in the lower panel showed similar results.

The rather widespread scatter of plotted points in this figure would indicate a rather inconstant system. This seems to be reflected in the transmission times of the pulses plotted, which varied from 55 to 79 msec. in the radial and 56 to 125 msec. in the femoral artery for *subject 2* and from 59 to 90 msec. in the radial and from 60 to 117 msec. in the femoral artery for *subject 1*.

The decrease in pulse-wave velocity to the femoral artery may also be related to the decrease in central pulse pressure amplification at this site during the Valsalva maneuver. Hamilton and associates (6) have proposed the concept of a net blood pressure during the Valsalva maneuver, which is the actual recorded intraluminal pressure minus the intrathoracic pressure. Wezler and Knebel (7) have pointed out that with reduction of net blood pressure in the Valsalva maneuver there is decreased distention of the thoraco-abdominal arterial walls, accounting for the decrease in pulse-wave velocity in these arteries. With

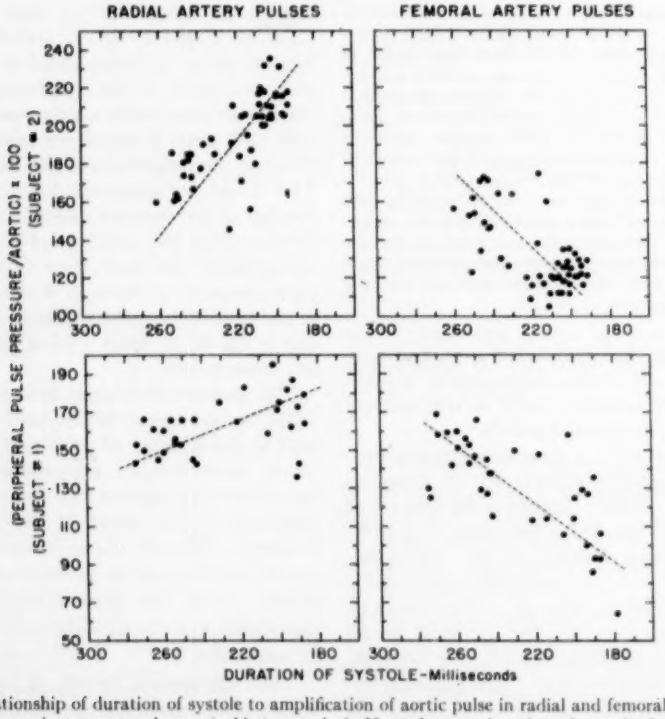

FIG. 4. Relationship of duration of systole to amplification of aortic pulse in radial and femoral arteries during Valsalva maneuver in two normal men (*subjects 1* and *2*). Note that, as duration of systole decreased, relative pulse pressure in radial arteries tended to increase, while that in femoral arteries decreased.

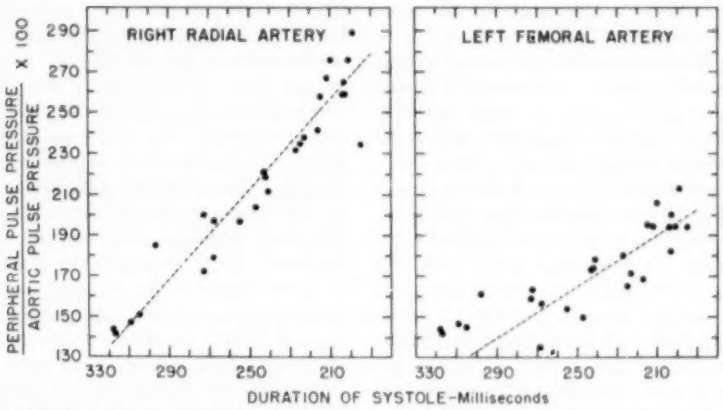

FIG. 5. Relationship of duration of systole to amplification of aortic pulse in radial and femoral arteries during a prolonged expiration (*subject 2*). Note that, as duration of systole decreased, relative pulse pressure in both radial and the femoral artery increased.

increasing Valsalva pressures the centrifugal tension on these arteries might approach zero. At the reduced pulse-wave velocity the effective length of the arterial segment is increased (or decreased in the resonant frequency). Thus it might be that, as central systolic time was shortened during the Valsalva maneuver, the resonant frequency was exceeded for the femoral aortic segment, and that decreasing amplification resulted.

Figure 5 shows pulses from *subject 2* plotted in a similar manner during prolonged expiration. In the supine position the duration of systole at the beginning of the procedure was longer (330 msec.) and the amplification of the central pulse pressure in the radial artery less (140%) than during the 40-degree head-up tilt in this subject (fig. 4). The peak amplification in the radial artery during prolonged expiration was higher (290%) than during the Valsalva maneuver at 200 msec. duration of systole.

During prolonged expiration, femoral pulse pressure amplification of the central pulse pressure, in contrast to the Valsalva maneuver, also rises to a peak amplification of 210% at 200 msec. duration of systole. Transmission time varied from 52 to 73 msec. to the radial artery and from 49 to 87 msec. to the femoral artery. Although the pulse-wave velocity to the femoral artery was also slightly diminished during the procedure, it was much greater than during the Valsalva maneuver. Thus the resonant frequency of the aortic femoral system was presumably greater than during the Valsalva maneuver so that amplification may be possible at the same duration of systole. In addition, as seen in figure 1, there was much less damping, as evidenced by the undulations following the primary peak. The heart rate was increased by 20 beats/min. above the Valsalva level and a different type of resonant phenomenon could be demonstrated in which the systolic build-up pressure was superimposed on an already rising peripheral pressure wave caused by vibrational energy remaining from the previous systole.

Figure 6 shows the relationship of duration of systole to central pulse-pressure amplification in the radial and brachial arteries of the same arm during a Valsalva maneuver carried out in the 40-degree tilt position. The subject in the top panels started with a very low radial pulse pressure amplification of only 110% at rest. This progressively increased with the shortening of systole during the Valsalva maneuver to a maximum of 180% at 200 msec. duration of systole. The subject in the lower panels began at a high level of 170% amplification in the radial artery and rose to 250% at 230 msec. duration of systole. In both cases peak amplification of the radial pulse is higher than peak amplification of the corresponding simultaneous brachial pulse. Brachial transmission times for *subject 4* varied only from 35 to 50 msec., and the corresponding radial transmission times varied from 54 to 67 msec. Transmission times for *subject 3* for the pulses plotted were from 38 to 60 msec. for the

brachial and from 52 to 77 msec. for the radial arterial pulses.

Figure 7 shows pulse data plotted in a similar fashion in which systolic times were shortened to extremely small values. In these two subjects during the Valsalva maneuver, as systolic times decreased beyond 200 msec., there was a progressive reduction in pulse-pressure amplification. The results resemble the frequency-response curve of a simple underdamped manometer system. In an attempt to fill in the left side of the curve, pulse data from the same individual during various physiologic states were included as indicated. The inconstancy of the system over this variety of physiologic conditions is well demonstrated by the wide spread of plotted points.

During the respiratory maneuvers outlined, the amplification of pulse pressure peripherally occurred despite the fact that the build-up time became progressively prolonged peripherally. This argues strongly against the hypothesis that the increase in amplitude is caused by a discrepancy in the velocities of the peak and the onset of the pulse wave, the peak overtaking the onset, so to speak (18). Here, with the reverse being the case, maximal amplification of central pulse pressure was obtained. The hypotheses relating the cause of peripheral pulse amplification to the natural frequency of oscillation of the arterial system or to the fusion of the reflected wave with the incident wave are practically interchangeable (19). A single impulse or wavelength, even under optimal conditions, can produce merely a doubling of amplitude in a simple system (17). The resonance theory of Frank best explains the phenomena observed during the Valsalva maneuver. However, as indicated in table 3, the pulse peaks and waves in the radial

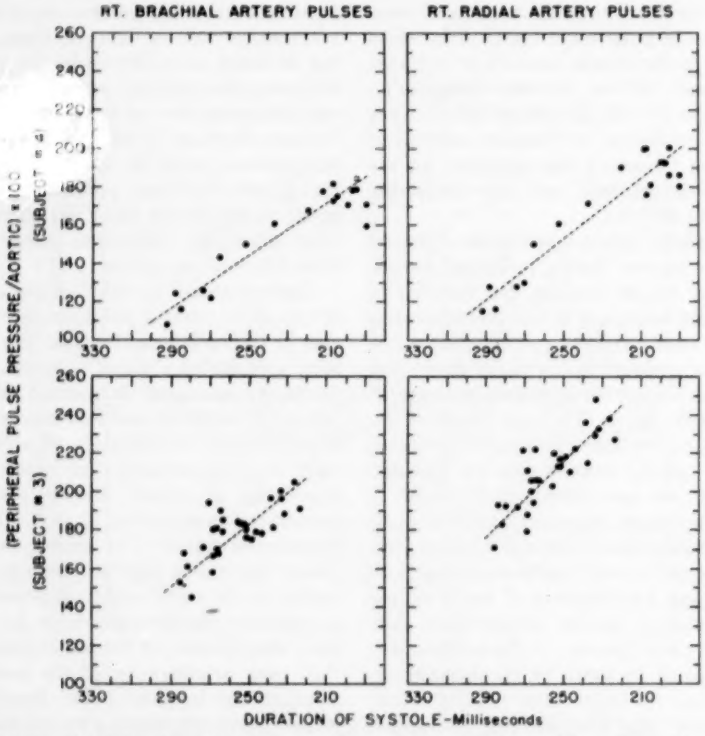

FIG. 6. Relationship of duration of systole to amplification of aortic pulse in right radial and brachial arteries during Valsalva maneuver in two normal men (*subjects 3* and *4*). Note that, as duration of systole decreased, relative pulse pressure in radial and brachial arteries increased in a similar manner.

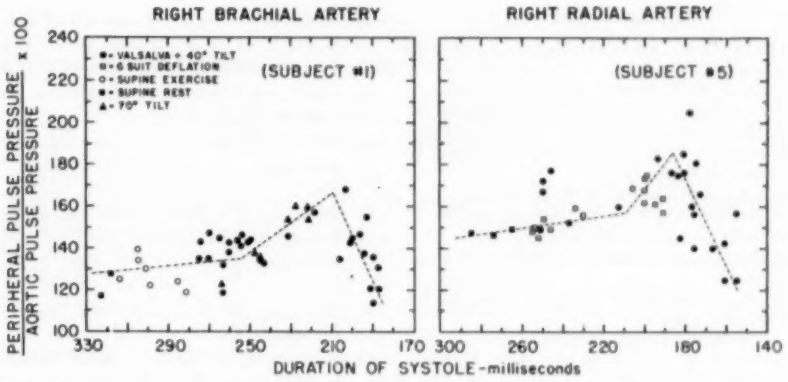

FIG. 7. Relationship of duration of systole to amplification of aortic pulse in brachial and radial arteries of two normal men (*subjects 1* and *5*). Note that, as duration of systole decreased, relative pulse pressure in radial and brachial arteries tended to increase up to a peak value ('resonant frequency'?) and then decreased when systole shortened still further. Note the similarity of this 'frequency-response' curve to that of a classic frequency-response curve of a simple underdamped instrument.

and brachial arteries in the same arm were not absolutely in phase as one might expect in a system resonating as a unit (15), although there was a progressive increase in amplitude peripherally (table 1), and central and peripheral mean pressures were almost identical.

The phenomenon of resonance is a physiologic curiosity and probably occurs more particularly in the longer arterial segments during conditions of stress with rapid heart rates and shortened systolic time. A similar phenomenon has been described in the central pulse of dogs in a state of shock (20). It is possible that resonance in the arterial system, besides being a curiosity, may also have physiologic significance. Flow would presumably be decreased in a resonating system. Resonance in the long, nonvital arterial segments during conditions of acute cardiovascular stress, therefore, may conserve blood for the short, vital arterial segments, which include the coronary, renal, splanchnic and carotid circulations. Resonance in the pulmonary circulation would be a serious disadvantage, since the whole cardiac output must go through it. The extremely short pulmonary arterial system probably precludes resonance in this system at heart rates possible in man.

SUMMARY

Simultaneous recordings of central and multiple peripheral pressure pulses were obtained in five healthy male subjects during performance of Valsalva maneuvers in the 40-degree head-up tilt position and prolonged expiration maneuvers. Striking beat-to-beat changes in the contour of central and peripheral pressure pulses were obtained and especially in the relationship between central and peripheral pulses generated by the same heart beat. Peripheral pulse pressures more than double aortic pulse pressures were obtained, peripheral dicrotic pressures dipping down to below end-diastolic pressures so that the general contour of the pulse resembled a simple sine wave. These changes in amplitude and form are in harmony with Frank's suggestion that the peripheral arterial system may resonate with the aortic pulse. The aortic pulse consists of two forcing impulses: a positive, the ascending limb; and a negative, the incisura. The distance between the two impulses is related to the systolic time. The optimal systolic time for resonance during the Valsalva maneuver could be determined (for any particular subject) by plotting systolic time against percentage increase in amplitude of the peripheral pulse, as in a classic frequency-amplitude response curve. As systolic time decreased from 300 to 200 msec., peripheral amplification of the pulse pressure in the radial and brachial arteries increased from 130 to more than 200%, with decreasing amplification thereafter.

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Posthypercapnic Hemodynamic Changes in Dogs[1]

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HYPERCAPNIA sufficient to produce symptoms of carbon dioxide intoxication occurred in many submarines during the Second World War (1). When personnel recommenced breathing fresh air after breathing air containing increased concentrations of CO_2, various symptoms including nausea, vomiting, headache and fainting followed. Earlier Itami (2) had noted that ventricular fibrillation occurred in cats and dogs, and Goldstein and DuBois (3) observed hypotension in humans following cessation of CO_2 breathing. Human deaths have been attributed to such posthypercapnic phenomena, as in the British submarine 'Thetis' disaster (4), and following routine surgical anesthesia (5). Dripps (6), and later Buckley and coworkers (5), established hypercapnia as the probable cause of 'cyclopropane shock.'

Miller and Brown showed in a series of paper (7–10) that in dogs a rapid return to air or oxygen following breathing of high CO_2 mixtures for periods of 2–4 hours, regularly led to a marked reduction in arterial pressure and severe alterations in the electrocardiogram, with ventricular fibrillation and death occurring in a high percentage of the cases. The only measures which they found to be effective in preventing these abnormalities were gradual reduction of the CO_2 concentration of the inhaled mixture over a 30-minute period or maintenance of arterial blood pressure by infusion of pressor agents during the first 15 minutes after returning to air breathing.

During a further investigation of posthypercapnic phenomena, Billings and Brown (11) demonstrated that loss of circulating blood volume did not significantly contribute to the immediate hypotension following rapid elimination of CO_2 in the dog.

The purpose of this investigation was to determine the hemodynamic changes responsible for the severe hypotension that follows prolonged or severe hypercapnia.

METHODS

Mongrel dogs weighing between 15 and 30 kg were anesthetized with intravenous thiopental (30 mg/kg), tracheotomy was performed and a no. 8 radiopaque Cournand catheter was passed into the right ventricle or pulmonary artery. In most of the dogs fluoroscopic guidance was used in passing the catheters and, where there was any doubt about the position of the catheter, its location was verified at necropsy. Polyethylene catheters were placed in either the carotid or femoral artery, or in both, and in the inferior vena cava via the femoral vein. A slow drip of heparinized saline kept the low pressure catheters patent when measurements were not being made.

Pressures were recorded from Statham Strain Gages with a Sanborn multichannel recorder which had electrical mean-determining circuits. These systems were calibrated at intervals during the experiment using mercury or butyl phthalate manometers.

Cardiac output was measured using the direct oxygen Fick procedure or the dye[3] dilution technique with direct continuous photokymographic recording of blood dye concentration (12). Each dye curve was calibrated using four blood samples which contained known dye concentrations, and for this purpose 20 ml of blood was drawn from the dog immediately before running the dye curve. Output was expressed as Cardiac Index using Meeh's formula (Surface Area = $11.2 \times \sqrt[3]{(\text{wt. in gm.})^2}$ to calculate body surface area. Total Resistance as used here is defined by the equation,

$$\text{Resistance} = \frac{\text{Mean Arterial Pressure (mm Hg)} \times 60}{\text{Cardiac Output (ml/min.)}},$$

where the arterial pressure is measured in the aorta.

A standard procedure of administering 30% CO_2 in O_2 for 2 hours was used in most experiments and any departure from this procedure is indicated. The 30% CO_2 in O_2 was administered from high pressure tanks of mixed gas through an open system. When other concentrations were used, mixtures were made continuously in a 100-liter spirometer and the CO_2 concentration in the bell was determined at frequent intervals with a Scholander analyzer (13). The Fischer

Received for publication October 10, 1955.

[1] This study was supported in part by funds provided under Contract No. AF 18(600)557 with the U. S. Air Force School of Aviation Medicine, Randolph Field, Texas.

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[3] A generous supply of Evans blue dye was provided by Warner-Chilcott Laboratories, St. Louis, Mo.

t-test was used to determine the significance of differences between means, with differences having a P value of 0.05 or less being considered significant.

RESULTS

Cardiac output was measured in 15 dogs using the oxygen Fick technique, following which 30% CO_2 in O_2 was administered for 2 hours. A second output measurement was made 10 minutes after switching the dog from the CO_2 mixture to 100% O_2 and a third 110 minutes later. In table 1 the data, with standard errors of the means of 15 dogs, are shown.

The immediate posthypercapnic hypotension was prevented in another series of four dogs by an intravenous infusion of *l*-norepinephrine during the first 10 minutes following the hypercapnia, with results as shown in table 2.

It may be seen from these tables that there was a marked fall in cardiac output following the hypercapnia. This posthypercapnia drop in output was not notably altered by administration of norepinephrine in four dogs. Moreover, it occurred in all of the 19 dogs, represented in tables 1 and 2, and was still present in 18 of these dogs 2 hours after they were returned to 100% oxygen breathing. Resistance increased progressively during the experiment, indicating an apparent vasoconstriction.

TABLE 1. HEMODYNAMIC CHANGES FOLLOWING TWO HOURS BREATHING 30% CO_2 IN 15 DOGS

	Control (before CO_2)	After 2 hr. on 30% CO_2	
		10 min.	2 hr.
Cardiac index (l/m² surface area)	3.5±1.5	1.8±0.6	2.0±0.3
Mean art. press. (mm Hg)	160±18.4	95±20.2	137±32.2
Resistance	4.0±1.4	4.9±3.1	5.3±1.3
Cardiac rate (beats/min.)	167±30.9	132±33.8	157±16.4

TABLE 2. EFFECT OF NOREPINEPHRINE ON POSTHYPERCAPNIC HEMODYNAMIC CHANGES

	Control (before CO_2)	After 2 hr. on 30% CO_2	
		10 min.	2 hr.
Cardiac index (l/m² surface area)	5.2	2.6	2.1
Mean art. press. (mm Hg)	159	139	125
Resistance	3.0	5.2	5.6
Cardiac rate (beats/min.)	185	132	172

Another series of experiments was carried out in which cardiac output was measured during the CO_2 breathing period and in the interval immediately following return of the dog to air or oxygen breathing. The dye method was used in these experiments in preference to the Fick procedure because in the latter it is necessary to draw blood samples over a 2-minute sampling period. This makes it difficult to accurately measure output during the period of relatively rapid change following hypercapnia. Moreover, the Fick method is difficult to apply when the dog is breathing CO_2 and also involves drawing a larger amount of blood from the dog. In the dye method, the blood which is drawn is immediately reinjected following the output determination.

The results obtained from 12 dogs are plotted in figure 1, expressed as percentage of control values in the case of arterial pressure, flow and resistance, and as mm Hg difference from control values in the case of venous pressure. Control values were considered as the value measured just prior to taking the dog off 30% CO_2. When the individual experiments were examined it was observed that cardiac output was lower after 2 hours of CO_2 breathing than it was immediately before CO_2 in all of the 12 dogs. The drop in output on withdrawal of 30% CO_2 also occurred in all 12 dogs.

Resistance was variable in the immediate posthypercapnic period (3–7 min.). In 6 of the 12 dogs resistance either fell or did not change. Within 14–20 minutes after changing from CO_2 to air, however, the resistance had risen (P = 0.023). This rise occurred mainly between 3 and 20 minutes after CO_2. Also it may be noted that the resistance had risen after 2 hours of breathing CO_2, as compared with the pre-CO_2 values (P = 0.007). In a total of 20 dogs for which blood pressure figures were available, posthypercapnic hypotension failed to develop in only one. Venous pressure changes are not statistically significant.

A reduction in cardiac output appeared to be the important factor responsible for the hypotension which followed elimination of

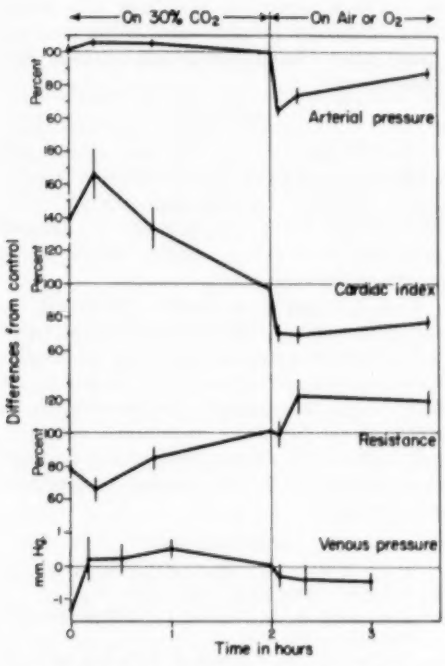

FIG. 1. Arterial blood pressure, cardiac index, total systemic peripheral resistance and venous pressure during and following 2 hr. of breathing 30% CO_2 in dogs. Vertical bars extend one standard error on each side of the mean.

CO_2 in these experiments. Changes in resistance were variable and could have been explained as resulting from normal vasomotor reflex responses to hypotension. In order to determine the response of the peripheral vascular system to a rapid reduction in arterial blood CO_2, experiments were performed in which systemic blood flow was maintained constant during the critical period following hypercapnia. This was accomplished using the technique of total cardiac bypass, with respiratory function being maintained by perfusion of an isolated dog lung. This method has been described by Campbell, Crisp and Brown (14).

The adaptation used here was briefly as follows. After the dog had breathed 30% CO_2 for about 1½ hours, positive pressure respiration with 30% CO_2 in O_2 was administered and the chest was opened. A catheter was inserted through the jugular vein so as to aspirate blood from both vena cavae and a cannula was tied into the carotid artery. Using two pumps, blood was aspirated from the veins and pumped through an isolated dog lung ventilated with 30% CO_2 in O_2. The oxygenated venous blood was then pumped into the carotid artery.

When this extracorporeal circuit was established both cavae were tied on the cardiac side of the pickup catheter and a clamp was placed on the root of the aorta leaving the heart totally bypassed. In both experiments flows sufficient to maintain femoral arterial pressure between 100 and 160 mm Hg were maintained. When a constant arterial pressure was attained the lung was switched from 30% CO_2 in O_2 to 100% O_2. This procedure, repeated five times in two experiments always resulted in a fall of 30–40 mm Hg in the arterial pressure of the dog. Readmission of CO_2 to the isolated lung was followed by a rise in pressure. Immediately following the first experiment the output of the perfusing pump was calibrated using blood drawn from the dog. This output was found to remain constant over a much greater range of resistances than obtained in the experiment.

DISCUSSION

In a series of 20 dogs, all but one developed a severe hypotension when returned to air or oxygen breathing after 2 hours of 30% CO_2. This result is in agreement with work reported by Billings and Brown (11) where a drop in blood pressure of approximately 50% was observed in all of 25 dogs returned to air after 30% CO_2. Such a change in pressure can occur only if one or more of the variables, blood flow, resistance or blood volume, decreases. It has previously been shown (11) that in the first 5 minutes after withdrawal of the CO_2, the circulating blood volume is elevated. During this same period blood flow, measured as cardiac output, fell in all of 15 dogs in the series where the oxygen Fick method of estimating cardiac output was used, and in 11 of the 12 dogs in which the dye technique was used. In all of the dogs in the dye series the output was greatly depressed between 14 and 20 minutes after CO_2 withdrawal. Since respiration was depressed in many of the dogs at the end of 2 hours of breathing 30% CO_2, and since

artificial ventilation was not used, the one discrepancy in this series could easily be accounted for by a delay in blowing off CO_2. Moreover in four experiments, shown in table 2, in which the resistance was increased by the use of norepinephrine, cardiac output still fell and to approximately the same degree. While venous filling pressure does decrease slightly at this time in some cases (5 of 10 dogs), it remains constant or rises in others, and in 7 of the 10 dogs for which measurements were made did not fall below the control pre-CO_2 value.

It appears probable, therefore, that a rapid reduction in arterial blood CO_2 tension after 2 hours of hypercapnia has a myocardial effect, the result of which is a reduction in cardiac output. Experiments previously carried out on dogs with totally denervated hearts indicate that this effect is not produced by a reflex mechanism.

The small rise in resistance observed during the immediate posthypercapnic period is not statistically significant. Inasmuch as resistance fell in 12 of the 27 dogs in the Fick and dye series, and since the normal response of dogs to hypotension would be a reflex increase in resistance, measurements of this variable were inconclusive. However, when flow was held constant and the dog perfused by an extracorporeal lung-pump system, resistance fell whenever CO_2 was withdrawn. Although a hematocrit change occurs at this time, which would be expected to decrease blood viscosity, the change is too small to account for the pressure change observed in these experiments (15).

Finally, it may be pointed out that we have not yet found a method for effectively preventing this posthypercapnic cardiovascular effect. While gradual lowering of the CO_2 concentration does appear to lessen the electrocardiographic abnormalities and the danger of ventricular fibrillation, as indicated by Miller *et al.*, severe hypotension may still occur. When the hypotension is prevented by using norepinephrine a fall in cardiac output is nevertheless observed. We have also found that adrenaline infusion used to prevent the hypotension also fails to prevent cardiac output decrease.

SUMMARY

Arterial pressure was observed to fall precipitously in 19 of 20 dogs switched to breathing 100% O_2 or air after breathing 30% CO_2 for 2 hours. This fall in pressure is produced primarily by a fall in cardiac output which occurs at the same time.

Post hypercapnic resistance changes in intact dogs were variable. This might be expected if the changes were the result of a decrease in resistance due to removal of CO_2 and of an increase of reflex origin. In experiments in which blood flow was maintained constant, reduction of CO_2 tension resulted in a decrease in resistance which was reversed by readministration of CO_2.

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Effects of Certain Local Anesthetic Drugs Upon Ventricular Tachycardia Resulting From Myocardial Infarction[1]

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A NUMBER of drugs with local anesthetic properties have proved to be effective in the suppression or prevention of ventricular ectopic arrhythmias arising from various experimental and natural causes. These compounds include cocaine (1), procaine (2–4), procaine amide (4–6), piperocaine (2), dibucaine (7, 8) of the cocaine derivative series and also quinidine (9, 10), which is not of this group but is chemically related to dibucaine in that the quinoline ring is common to both.

Of the synthetic local anesthetics listed, procaine amide (4) and dibucaine with phenobarbital sodium (7) have demonstrated ectopic impulse suppressor effect in ventricular tachycardia due to experimental myocardial infarction in sufficient degree relative to toxicity to offer promise of clinical usefulness, and procaine amide has gained wide acceptance for treatment of human ectopic arrhythmias arising from this and other causes. Procaine has exhibited some degree of ectopic suppressor effect in infarction tachycardia, but the effect is transitory and offers little probability of practical value for treatment of this persistent arrhythmia (4).

Tests of the effectiveness of an additional group of local anesthetic compounds in the control of ventricular tachycardia in dogs with acute myocardial infarction are to be described. The compounds used were tetracaine, Panthesine, lidocaine, piperocaine and hexylcaine.[3]

Tetracaine (11) and piperocaine (12) have proved to be widely useful for regional anesthesia. Panthesine (13, 14) is reported to have given good results with little toxic reaction following intravenous and infiltration application in a variety of painful conditions. Lidocaine, a recently developed local anesthetic, has been selected as an agent of low toxicity which possesses properties that make it suitable for injection and surface anesthesia (15).

Hexylcaine (16) has demonstrated local anesthetic effectiveness together with low toxic activity comparable to that of procaine. It has been observed to be more effective than procaine or procaine amide in preventing responses of auricular muscle to electrical stimulation (17).

TECHNIQUES

The methods of preparation of animals and of testing the effectiveness of antiarrhythmia drugs in dogs with myocardial infarction ventricular tachycardia have been presented previously (10). These procedures, therefore, will be described briefly. Under pentobarbital sodium anesthesia (30 mg/kg) and with aseptic surgical precautions, the anterior descending ramus of the left coronary artery of the dog's heart is dissected free for a distance sufficient to pass ligatures under it at the level of the distal edge of the left auricular appendage. A doubled ligature is then passed under the artery and cut, making two ligatures. A partial occlusion is produced by tying one ligature snugly, but not tightly, around the artery together with a 20-gauge hypodermic needle, and immediately withdrawing the needle. The second ligature is tied tightly around the artery after an interval of 30 minutes. By this two-stage technique, losses of animals by ventricular fibrillation during the

Received for publication September 6, 1955.

[1] This investigation was supported in part by research grant ✱H-1109 (C3) from the National Heart Institute, National Institutes of Health.

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[3] The tetracaine used was Pontocaine hydrochloride, manufactured by Winthrop-Stearns, Inc. Panthesine is manufactured by Sandoz Pharmaceuticals. The lidocaine used was Xylocaine hydrochloride manufactured by Astra Pharmaceutical Products, Inc. The piperocaine used was Metycaine hydrochloride manufactured by Eli Lilly and Co. The hexylcaine used was Cyclaine manufactured by Sharp and Dohme, Inc. These agents were generously contributed by the respective manufacturers for experimental use.

danger period of the first 10 minutes after abrupt occlusion are avoided. The chest is closed and the animal is given fluids and other routine postoperative care.

On the following morning, 16–20 hours after occlusion, and after the animal has completely recovered from anesthesia, a number of control electrocardiograms are made and testing with drugs is begun. At this time, the animals characteristically have a persistent ectopic ventricular tachycardia which exhibits only minor changes in frequency from hour to hour. If allowed to run its course without treatment, the ventricular tachycardia usually continues for 2–4 days. In about 5–10% of the animals, the ectopic activity is not sufficiently rapid nor sufficiently constant to be suitable for testing. The principal testing is done on the first postoperative day when ventricular activity is most intense and most difficult to control. In some animals, testing is resumed on subsequent days. The term 'test' is used to denote all of the doses of the test drug and all procedures and observations performed during any one day of testing.

Phenobarbital sodium, 25–40 mg/kg, was administered to a majority of the animals either prior to the beginning of testing with a local anesthetic agent or, in fewer trials, during the course of the test after some of the test drug had been administered. Phenobarbital was given for two reasons: *a*) for sedation to help keep the animal quiet during intravenous infusion and *b*) to prevent or reduce the severity of toxic reactions to the local anesthetic drugs administered (7). Phenobarbital also has some ectopic impulse suppressor effect when used alone in sufficient doses. Doses as large as about 60 mg/kg have significantly reduced the frequency of ventricular tachycardia in some trials, and abolished low frequency ectopic activity for short periods (18). In a large number of experiments in different studies phenobarbital alone in doses of 25–40 mg/kg has exhibited relatively little effect upon ventricular tachycardia resulting from myocardial infarction.

RESULTS

All of the local anesthetic compounds tested were capable of reducing or abolishing ectopic activity for certain periods of time if used in adequate amounts and at sufficient rates of administration. Ectopic suppressor dosage of each of the drugs also produced toxic side reactions, most commonly convulsive movements or vomiting. There were differences in degree of ectopic impulse suppressor effect by the different local anesthetic agents relative to toxic reactions. Phenobarbital was less effective in preventing toxic effects of tetracaine, hexylcaine, lidocaine and piperocaine than had previously been found in experiments with dibucaine (7).

Table 1 contains data from selected experiments, chosen to represent the usual effects of the various compounds used. Each experiment was charted in detail to facilitate thorough study of duration and degree of reduction of ectopic activity in relation to periods of administration of the drugs employed, and the toxic reactions. One of the charts is reproduced in figure 1 for illustration of the method of graphic representation used.

Tetracaine was used in six tests in four dogs with ventricular tachycardia. In each test, phenobarbital, 25–40 mg/kg was used for sedation. In five of the six tests, the administration of sufficient tetracaine to markedly reduce the ectopic frequency resulted in vomiting or convulsions or both. The total dose of tetracaine used in a test varied from 8 to 33.5 mg/kg infused in divided portions over total periods of 2–6½ hours. The blood pressure was well maintained throughout the procedures (fig. 1). It was possible to restore normal rhythm without evidence of toxicity in one test only. In this case, a relatively low frequency ectopic arrhythmia (50–100/min.) was present at the beginning of the test. The control ectopic rates in four of the five remaining tests ranged between 170 and 230/min.

Panthesine was administered in three tests performed on two dogs. Phenobarbital was used for sedation in two of the tests, the dose being 40 and 25 mg/kg. The total dose of Panthesine infused during a test was 33–52 mg/kg. The over-all period of administration was 1.3–5.5 hours. Panthesine markedly reduced or abolished ectopic activity in all three trials. In two tests there were no visible signs of severe toxic reactions. In the test without phenobarbital, the animal exhibited coarse tremors of the head, and eventually voided urine tinged with blood. This dog had been tested with Panthesine on the previous day with good ectopic control during infusion periods, and without toxic signs. Marked suppression of ectopic activity could be maintained with Panthesine only during the period of its administration. Upon cessation of the infusion, the tachycardia recurred promptly.

Hexylcaine was used in seven tests in four dogs. The total dose of hexylcaine infused during a test varied from 40 to 120 mg/kg and the total period of administration was 2.25–6 hours. In five tests, infusion at rates sufficient to markedly reduce or stop ectopic

TABLE 1. EFFECTS OF LOCAL ANESTHETIC DRUGS AND PHENOBARBITAL UPON VENTRICULAR TACHYCARDIA RESULTING FROM MYOCARDIAL INFARCTION

Drugs and Doses, mg/kg		Period of Admin., hr.	Ectopic Rate		Duration Reduc. to ½, hr.*	Comments
			Control	Minimal		
Phenobarb. Tetracaine	25 8	4.75	170–180	5	1.5	2 infusions, each 4 mg/kg in 45 min. Interruption by vomiting. Ectopic suppression brief after administration stopped
Phenobarb. Tetracaine	40 26	5.5	210–230	0	4.5	Ectopic impulse suppression by infusion at rates that produced vomiting and convulsions. Blood pressure initially raised and then maintained. Chart shown in fig. 1
Phenobarb. Panthesine	40 52	5.5	190–200	0	4.0	Marked reduction of ectopic rate only during periods of infusion. Head tremors; restless
Phenobarb. Hexylcaine	25 90	6.0	250	0	1.5	Vomiting and convulsions upon admin. of ectopic suppressor doses
Hexylcaine	52	2.25	150–160	0	1.5	Same animal; 2nd postoperative day. Ectopic suppressor effect only during infusion
Phenobarb. Lidocaine	40 103	6.2	200	0	7.0	4 infusions lidocaine, 43, 25 and 35 mg/kg. Convulsions during last infusion
Lidocaine	50	3.75	80–140	0	2.0 +	Same animal. Erratic ectopic suppression; convulsions
Phenobarb. Piperocaine	50 31.7	6.5	150–180	15	1.0	Extreme restlessness before reduction of ectopic activity. Highest ectopic rate near end of infusion. Died a few hours later

* Duration reduc. to ½ = total time during and immediately following administration of test drugs during which ectopic rate was maintained at levels less than one-half of control rate.

activity produced vomiting or convulsions or both. In one test on the second postocclusion day, ectopic activity was well controlled for 3 hours without any toxic manifestations. In one other animal, good control was achieved with toxic signs limited to head tremors.

Lidocaine was tested in six animals, a total of eight tests being performed. In two of the tests lidocaine was used alone. Both of these were second postocclusion-day tests with relatively low ectopic rates. The ectopic activity was well controlled in each case by total doses of 50 mg/kg in divided infusions. Phenobarbital, 25 or 40 mg/kg, was used in the other six tests. Effective control of the ectopic arrhythmias was achieved in five tests. Convulsive activity occurred in every test. Vomiting occurred only once in the experiments with lidocaine. In the one animal in which restoration of normal rhythm was not brought about, administration of lidocaine was more rapid than in the other trials, 75.5 mg/kg having been infused in 70 minutes. This test was terminated by death of the animal. In other trials the rate of infusion usually was 40–50 mg/kg/hr., and the total dose, divided between two or more periods of administration, was 75–176 mg/kg. Blood pressure was well maintained in all animals in which it was measured, except the one fatality, and in that case pressure showed little change until about five minutes before collapse.

Piperocaine was used in one dog. A severe degree of restless activity occurred during infusion, even after the administration of phenobarbital, 50 mg/kg (2 doses, 25 mg/kg each, 1.1 hr. apart), but there were no convulsions nor vomiting. Some ectopic impulse suppressor effect was evident during the midportion of the test but it was of short duration. With additional infusion, the ectopic rate increased, reaching a frequency higher than the

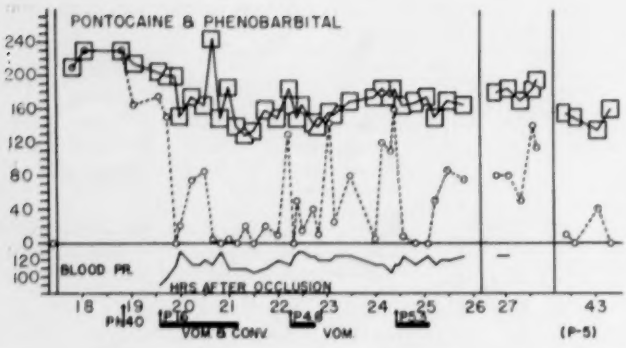

FIG. 1. Effects of tetracaine (Pontocaine) in dog with ventricular tachycardia associated with myocardial infarction. *Circles*, ectopic rate; *squares*, total heart rate; *Ph 40* = phenobarbital sodium, 40 mg/kg; *P 16* = tetracaine, 16 mg/kg infused during period indicated by length of black bar as measured by time scale along abscissa. Other infusions indicated similarly.

control before the test was terminated. The dog died during the following night.

DISCUSSION

Mechanism of Action of Ectopic Suppressor Agents. It is logically to be expected that the mechanism of inhibition of the discharge of ectopic impulses is intimately related to the nature of the excitatory factors that cause the generation of ectopic impulses.

Previously reported experiments (19) have shown that ischemia of an area of ventricular myocardium results in the migration of potassium from the ischemic cells to extracellular fluid in sufficient quantity to significantly elevate potassium concentration in coronary venous blood draining the region. The local elevation in potassium concentration exhibits quantitative fluctuations with time that parallel roughly the fluctuations in ectopic activity, i.e. the development, rise to maximal frequency and subsidence of ventricular ectopic impulses that result from myocardial ischemia and infarction. The injection of potassium chloride into coronary arteries of hearts without ischemia also produced ectopic arrhythmias. These observations, considered together, are regarded as evidence that the ectopic events which result from regional ischemia and infarction are produced, in part, by the influence of local elevation of extracellular potassium concentration upon neighboring cells that remain capable of responding.

It is well known that potassium is lost from cells of nerve and muscle during depolarization whether it be due to excitation, oxygen lack or depolarizing chemical agents such as Veratrin. Cocaine and a variety of synthetic local anesthetic compounds reduce the rate of transport of potassium across cell membranes under a variety of experimental conditions, including lack of oxygen. This action has been studied in detail and forms the basis of an hypothesis to explain the mechanism of local anesthetic action (20–23).

Depolarizing action of excess extracellular potassium is reduced by local anesthetics (21). The findings (19) which indicate that local potassium liberation during myocardial ischemia and infarction is a significant factor in ectopic excitation point to a corollary that the ectopic suppressor effect of local anesthetic compounds is a consequence of diminution of cell-membrane permeability to potassium by these agents. Thus, one probable factor in ectopic excitation would be quantitatively reduced. Certain experimental findings indicate that additional excitatory factors may contribute to the production of ectopic arrhythmias in infarction. Some that have been proposed are sympathomimetic amines liberated from autolyzing muscle (24) and injury potential (25). Neither has been fully evaluated as yet, but the injury potential probably is not a factor of sufficient relative importance to determine the pattern of the arrhythmias with time. No phasic fluctuations of injury potential with time which correspond to the phasic fluctuations in ectopic arrhythmia that characterize dog ischemia and infarction arrhythmias (26) have been detected.

Practical Considerations. The therapeutic usefulness in persistent arrhythmias of drugs with demonstrated ectopic impulse suppressor properties is subject to limitations imposed by toxic reactions produced in intact unanes-

thetized or moderately sedated animals by the relatively large dosages that are required to suppress ectopic pacemakers. The duration of control of the arrhythmia after it is achieved and after drug administration is stopped also merits consideration. When severity of toxic reactions and duration of control are taken into account, results with tetracaine, hexylcaine and lidocaine compare unfavorably with results previously obtained with intravenous quinidine compounds, procaine amide and dibucaine with phenobarbital. Panthesine may deserve more extensive trial, though its duration of effect after cessation of infusion was brief. Piperocaine perhaps should not be judged upon results of one test, but the pattern of response was unfavorable.

SUMMARY

Tests of the ectopic suppressor effectiveness of five local anesthetic agents, and of toxic manifestations induced by them, were performed in dogs with ventricular tachycardia associated with myocardial infarction. The agents tested were tetracaine (Pontocaine), Panthesine, lidocaine (Xylocaine), hexylcaine (Cyclaine) and piperocaine (Metycaine). All of these compounds proved to be capable of reducing or abolishing ectopic activity for certain periods of time if used in adequate amounts and at sufficient rates of infusion. Convulsive movements and vomiting resulted from ectopic suppressor dosage with tetracaine, Cyclaine and lidocaine even with phenobarbital sedation. Panthesine was less toxic, but produced some head tremors when used alone. The duration of antiarrhythmic action of Panthesine was brief after infusion was stopped. In the one dog tested with piperocaine, the drug first reduced the ectopic frequency and then increased it beyond the control level. This pattern of response has previously been found unfavorable. In the discussion, the possible interrelationships that may exist between the ectopic suppressor effects of local anesthetic drugs and reduction of permeability of cell membranes to potassium by them are pointed out.

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Influence of Venous Occlusion and Procaine Block on Peripheral Vascular Responses to Frostbite

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PREVIOUS investigations (1, 2) have demonstrated that the vascular reactions to frostbite are not limited to the site of injury but are distributed widely in remote peripheral vascular areas. It was also noted that there is a discrepancy in the time factor between the onset of frostbite and the appearance of responses in peripheral circulation. This observation, coupled with the fact that pretreatment with alcohol prevents these reactions, led to the belief that peripheral vascular responses to frostbite are elicited by an injury substance produced at the frostbitten site, carried through the blood stream and acting on the vasomotor center. Although toxemia theories have been widely proposed, there is a lack of clear experimental evidence to link a toxic factor with the vascular responses to frostbite. Wiggers (3) traces the history of such theories to their inception.

The acknowledged action of heat and cold on nerves and the compensatory vascular reactions produced offer strong support for theories of neurogenic involvement in frostbite. Greene (4) and Lange *et al.* (5) are among a number of investigators who have proposed that the vascular responses to frostbite are due, in whole or in part, to nerve stimulation. Hemingway and French (6) propose that the vasomotor responses to thermal stimuli are under a coarse central nervous system control regulated by brain temperature, and a fine reflex control. That the vascular responses to frostbite are due solely to coarse control by the vasomotor centers seems unlikely in view of the findings of Lewis and Gerstner (7) that the core temperature varies only slightly after cold injury. In addition, the time lapse between the onset of cold injury in the leg and the appearance of peripheral responses is not compatible with any theory of reflex control alone.

Received for publication July 7, 1955.

An analysis of previous work in the field strongly indicates the production of a chemical injury substance following frostbite. However, the possibility of nerve involvement has received considerable attention. The present investigation is an attempt to evaluate both theories by means of direct experimentation.

MATERIAL AND METHODS

The golden hamster, *Mesocricetus auratus*, was used throughout the investigation. Each animal was anesthetized by an intraperitoneal injection of 0.25 cc of Nembutal containing 50 mg of pentobarbital/cc. The cheek pouch was everted and prepared according to a previously described technique (1, 2). The membranous pouch was spread over an optical glass block on a temperature stage and continuously irrigated with physiological saline (37–39° C) during the entire period of observation.

One hind limb of each hamster was depilated to facilitate the induction of cold injury. Finely granulated carbon dioxide ice was applied to the limb in a boot of aluminum foil. An approximately equal area of the limb below the knee was exposed in all animals. Complete frostbite was achieved after an exposure of 5 minutes, as indicated by the appearance and rigidity of the limb.

General occlusion of circulation, into and out of the exposed portion of the limb, was obtained by means of a tourniquet of stout cord applied above the knee. After the tourniquet was secure the extremity was exposed to the freezing boot. It was removed within 30 minutes after the frostbite had been produced.

Nerve blockade of the hind limb was accomplished by means of subcutaneous and intramuscular injections of a 2.0% solution of Novocain (procaine hydrochloride, Winthrop-Stearns, Inc.) in unbuffered saline solution. An initial dose of 15 mg was injected subcutaneously along the course of the femoral nerve and intramuscularly over a wide area in the region of the sciatic nerve and the lumbosacral plexus. That an effective block had been obtained was demonstrated by the absence of a response to a thermal stimulus (60° C). In the nonprocainized limb the response consisted of a quick and pronounced withdrawal of the limb from contact with the stimulus. The nerve block was maintained by additional injections of 15 mg Novocain at 30-minute intervals.

Observations were made on animals divided into three primary categories: *a*) frostbite controls, *b*) animals subjected to occlusion of circulation and *c*)

animals subjected to nerve blockade. In the two latter groups the effects were observed after frostbite and in nonfrostbite controls.

RESULTS

Frostbite Controls. *Peripheral responses.* Following exposure of the hind limb to the cold source peripheral vascular responses in the cheek pouch occurred in three phases. The initial freeze phase responses were observed within 30 seconds after application of the freezing boot and persisted throughout the duration of the freezing process. This phase is characterized by a marked increase in the rate of flow in all vessels in the vascular bed. The arterial flow appeared to increase within 5 seconds, with venous flow increasing within 30 seconds. Capillaries showed an initial increase in flow followed by a decrease in 3 or 4 minutes. The second or transitional phase was observed within a few minutes after the onset of thawing. It is characterized by a gradual decrease in the rate of blood flow to the control level. The duration of this phase varies, depending upon the initial appearance of constrictions in the arterioles. The third phase of vascular responses, the constriction phase, began in 15–20 minutes from the time of removal of the freezing boot and was most marked after 25–30 minutes. During this period marked constrictions were apparent in arteries and arterioles. Most noticeably affected were the smaller arterioles and the precapillaries which sometimes contracted along their entire course. At this time the capillary beds were largely closed and the number of functional vessels was greatly reduced. Venous flow was extremely sluggish, with large numbers of leucocytes accumulating at the periphery of the stream. At times, definite coatings of cells (either leucocytes, platelets or both) were apparent on the walls of venous channels, narrowing the actual area of flow to $\frac{1}{3}$ or $\frac{1}{4}$ of control diameter. Partial stasis occurred over widespread areas of the pouch, although no intravascular agglutination occurred. This was indicated by periods of temporary release when the capillaries opened partially to allow slightly increased flow for brief intervals. The duration of the third phase was variable. In some cases return to normal conditions was observed within 45 minutes after thawing had begun, while in other cases arterial constriction persisted throughout the 2-hour period of observation following the onset of thaw.

When the freezing process was increased to 10 minutes no sigificant difference was observed in the initial or transitory phases of the response, but the third phase was delayed. Constrictions did not appear until 15–20 minutes after the onset of thaw and were not marked until 40–45 minutes after removal of the ice from the limb.

Rapid rewarming of the frozen part at 42°C produced changes in both 2nd and 3rd phases of the response. The transitional phase was considerably shortened and a rapid decline in the rate of blood flow was apparent within a few minutes after immersion of the injured limb in the warm water. The degree of arteriolar constriction was reduced in the 3rd phase and the larger vessels were unaffected. Little if any capillary closure or venous stasis was observed.

Local effects. Following removal of the freezing boot the limb appeared whitish, cold and rigid. After a 5-minute exposure, thawing was completed within 20–25 minutes after removal of the boot, judging by the restoration of color and warmth to the limb. The affected parts became more reddened and warmer than normal, and the latter stages of thaw were accompanied by the onset of edema. The swelling reached its maximum within 2 hours after removal of the ice. The limb volume increased from a mean control value of 0.52 cc to a volume of 0.96 cc.

Between 24 and 48 hours after freeze injury the affected parts assumed a cyanotic hue and showed signs of tissue atrophy. Gross gangrene appeared within 3 days. All animals suffered complete loss of the exposed portions of the limb. However, they appeared normal in reactions and apparently suffered no discomfort beyond the irritation resulting from the necrotic tissue. When this had been sloughed off the animals showed no further signs of discomfort.

Influence of Circulatory Occlusion. *Controls.* Tourniquets applied above the knee and allowed to remain for 1 hour produced no changes in peripheral circulation, either in the period of circulatory occlusion in the limb or following release of the tourniquet. Locally the temperature of the limb decreased slightly and a slight

cyanotic coloring was evident within 30 minutes. Upon release of the tourniquet the limb reddened slightly, but there was no evidence of any edema.

Frostbite. The peripheral circulatory responses to frostbite were altered by circulatory occlusion of the limb, whether the tourniquet was applied before exposure or following exposure but before removal of the freezing boot. However, not all phases of response were affected.

The initial freeze phase was unaltered. The usual increase in the rate of blood flow was evident in all channels. The transitional phase was unaffected except that its duration was prolonged until the tourniquet was released, 30 minutes after frostbite.

The constriction phase did not become evident until the tourniquet was released. Within 5 minutes of the removal of the circulatory occlusion all animals began to show a gradual abatement in the rate of flow, climaxed within 30 minutes by the usual conditions of capillary closure and partial venous stasis. Constrictions appeared in precapillaries and arterioles within 6 minutes after removal of the tourniquet. Within 20 minutes these constrictions extended into the larger vessels of the arterial tree, becoming more widespread and more severe than any seen in frostbite controls.

Local effects. The frozen limb appeared to thaw within the usual 30 minutes even though circulation was still occluded. Before release of the tourniquet the limb was deep scarlet in color, but the edema and warmth, normal sequelae of the thawing process, were absent. Following release of the tourniquet the intense color was gradually reduced until at the point of maximum edema only a slight reddening was evident.

Influence of Nerve Blockade. *Controls.* Novocain nerve blockade of the limb produced no changes in the peripheral vascular bed in the cheek pouch. The limb was likewise unaffected. That the sensory nerves had been blocked was demonstrated by the absence of a response to a heat stimulus (60°C) which produced a marked withdrawal of the nonprocainized limb from contact with the stimulus.

Frostbite. Nerve blockade in the hind limb produced slight changes in the remote peripheral vascular responses to frostbite. The initial freeze phase was absent. There was no evidence of an increased rate of blood flow following frostbite. Due to the absence of the initial acceleration of flow the transitional abatement response was absent. Rate of flow and caliber of the vessels remained at the control level.

There were no variations in the constriction phase in the nerve-blockaded animals. All of the characteristic features of the constriction phase in the controls were apparent in the Novocain-treated animals.

Local effects. Thawing was normal in the procainized limb. The typical redness of the limb was not as apparent as in the controls but there were no significant changes in the increase of limb volume or in the amount of tissue lost as a result of the cold injury.

DISCUSSION

It is evident that any discussion of frostbite injury must consider both the local and systematic sequelae. Local effects reported here were confined to obvious macroscopic changes in the color and volume of the limb and the extent of tissue damage. However, the local vascular picture has been the object of many studies. Rosenfeld *et al.* (8) and Lewis and Gerstner (7) offer evidence for the existence of widespread postthaw vasodilation in the injured area. Though there exists a controversy concerning this view, Kreyberg (9) affirms that vasodilation, and not vasoconstriction, is the serious circulatory involvement in frostbite. The local vascular picture, therefore, is actually one of vasoconstriction during exposure, return toward normal conditions during thaw and vasodilation following thawing. Since the three phases of responses in the cheek pouch are directly opposed to this, it suggests that the remote peripheral responses are compensatory, that vasodilation and subsequent blood pooling in the limb result in reduced blood volume and compensatory vasoconstriction in remote peripheral vascular beds. The results of this investigation offer evidence that both neurogenic and toxemic circulatory factors are involved in producing these responses.

The application of dry ice to the limb produces a local vasoconstriction that is undoubtedly of reflex nervous origin. Since Novocain

nerve blockade of the limb prevents peripheral manifestations of this initial response a nervous involvement, in addition to the simple local reflex, is indicated. The time factor, the type of response and the absence of response in the procainized animal seem to indicate that the freeze phase of peripheral vascular response is of neurogenic origin.

The transitory phase is difficult to evaluate but apparently it may be due to either a neural mechanism or a natural return toward normal conditions following the removal of a stress or a combination of both factors. As a manifestation of nervous control, it would be a typical response to a slow decline in the strength of stimulus as the frozen area gradually thaws. Since the freezing produces an effective denervation of the affected area, whatever impulses could be transmitted to the vasomotor center must arise from the undamaged nerves proximal to the injured area. It seems more likely that the transitory phase is merely a natural and gradual tendency toward normal conditions following removal of the stress. This phase, however, is relatively unimportant. As the name implies, it is a simple transition between the two significant phases.

The constriction phase, the most prominent of the three stages of response, is primarily due to a chemical mediator within the circulatory system. The absence of peripheral vasoconstrictions when venous return from the affected limb was occluded by tourniquet, and the appearance of these constrictions when the tourniquet was released indicate the presence of a blood-borne vasopressor substance, probably produced by the injured tissue. The fact that the vasoconstrictions occurring after the release of the tourniquet are more widespread and more severe than any seen in the frostbite controls might be taken as a further indication of the presence of a vasopressor substance. In the frostbite controls any substance emanating from the injured area is dissipated gradually as it is produced, whereas circulatory occlusion by means of a tourniquet allows for the sudden release of larger amounts of injury substances, and presumably the peripheral circulatory effects are more severe.

If on the basis of the observations reported here the presence of a toxic metabolite may be assumed, its nature is open to much speculation. Lewis (10), who proposed the release of an H-substance from frozen tissue, and Crismon *et al.* (11), who reported the isolation of a vasodepressor material from the edema fluid in frozen extremities, offer evidence of a vasodepressor substance. These findings would seem to account for the reported vasodilation in the injured area. It would also make it appear that the widespread peripheral vasoconstrictions are merely compensatory reactions consequent upon the local vasodilation and blood pooling in the limb and probably in the splanchnic vessels. In contrast to this, plasma from frostbitten animals, when applied topically to the cheek pouch, produces nothing but constrictions. Page (12) reported the same reaction with blood from animals subjected to traumatic, hemorrhagic and burn shock.

It seems logical to assume that this vasopressor substance exerts an influence on the vasomotor center or acts directly on vascular muscle elements in the remote peripheral areas. It is not likely that constrictor impulses emanating from the vasomotor center would elicit a response in the injury area since the freezing process constitutes a sympathectomy in effect. Direct action on the blood vessels in the injury area is probably inhibited by loss of vascular tone or the presence of a stronger vasodepressor mediator.

Observations made in this study also indicate that there may be a definite correlation between the duration of freeze and thaw processes and the amount of vasopressor substance released by the injured tissue. Severe peripheral vasoconstrictions following freezing of the limb for 10 minutes were not apparent until 45 minutes after the onset of thaw, as opposed to 25 minutes following a 5-minute exposure. It is significant that severe constrictions are observed at the time that thawing is apparently complete.

When the frozen limb was rapidly rewarmed the subsequent constrictions appeared no earlier than in the controls but were never too severe. Thus, the injury substance, which is released only as thawing is effected, is apparently produced in greater amounts as thawing is prolonged. Rapid rewarming, though it reduces the amount of vasopressor substance released and decreases the amount of tissue loss, is debatable as a therapeutic measure because greater harm

ensues if the metabolic demands of the tissues are increased before adequate circulation is established.

SUMMARY

The peripheral vascular responses to frostbite occur in three phases in the cheek pouch of control animals. The initial freeze phase is characterized by an increased rate of blood flow in all peripheral vessels. The transitional phase is marked by a gradual return to control conditions. The constriction phase is indicated by pronounced vasoconstriction and prolonged venous stasis.

When circulatory occlusion is induced by means of a tourniquet prior to freezing the limb, the first two phases are unaltered, having all of the typical features of the controls. However, the constriction phase is not apparent until the tourniquet has been released. The resulting constrictions are then of much greater severity than in the controls.

When nerve blockade of the hind limb is produced by means of procaine prior to freezing the limb the initial freeze phase is absent. There is no evidence of increased blood flow. Consequently the transitional phase does not occur. The constriction phase occurs without any variations from the controls.

It is proposed that the preliminary phase is due to reflex nervous reaction, the second phase is a natural trend toward normal conditions, and the constriction phase is brought about by a blood-borne vasopressor substance originating in the injured tissues.

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Alveolar Air Changes Following Inhalation of Carbon Dioxide During Exercise, and Calculation of Cardiac Output[1]

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ATTEMPTS to bring the alveolar air into equilibrium with the tension of carbon dioxide in the mixed venous blood have experienced many difficulties. The purpose of these investigations has usually been the determination of the tension of carbon dioxide in the mixed venous blood coming to the lungs, a figure useful for the indirect estimation of the circulation rate according to the Fick Principle. The chief difficulty has been in revealing any definite or sustained level of carbon dioxide suggestive of a tension equilibrium of this gas between the lung air and the venous blood obtainable within the recirculation time.

The following results are of interest in that they demonstrate changes in the alveolar air (at rest and during exercise) in a series of normal subjects breathing air and carbon dioxide mixtures, using a technique for the sampling of the alveolar air which differs in several respects from previous methods. Details have been discussed in an earlier paper (1), and additional experiments have also been reviewed in another communication (2).

METHODS AND RESULTS

Arterial and venous CO_2 pressures were determined by the method outlined below, and the CO_2 output measured by means of a spirometer. Resting arterial blood estimations were made concurrently in order to check the alveolar values, and provided a satisfactory comparison. These arterial blood samples were obtained with an indwelling plastic catheter in the brachial artery according to the technique described by Schnabel *et al.* (3), and have been reported separately (2). The mean of three alveolar readings was taken on both arterial and venous levels, it having been demonstrated previously that the variation of these readings is small, and only the mean is included in the table.

Since the apparatus has been described fully (1, 2, 4), only the following summary of the routine will be given. The accompanying diagrams will help to make the procedure clear. After resting in a constant temperature room for an hour in the recumbent position (each subject having had no breakfast) the arterial catheter was introduced. After a further 30 minutes three alveolar air samples were taken by the oral suction method at 2-minute intervals, preceded and followed by an arterial blood sample. As soon as this was completed (the subject all this time breathing through the apparatus, fig. 1, with the nose clipped), the tap connecting the 15% CO_2 mixture was turned in order to allow the

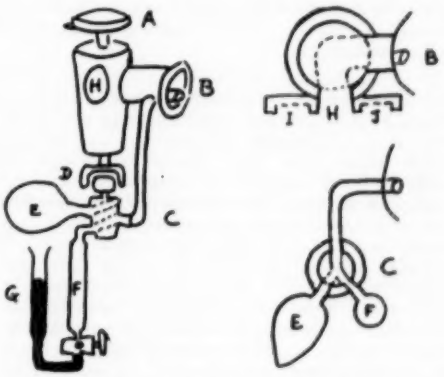

FIG. 1. *Left:* oral suction apparatus used for obtaining arterial and venous alveolar air samples; *right:* section through apparatus at level *B* and level *C*. *A*, sampling tap. This connects mouth to room air at one position; during the rest of a complete rotation the oral cavity is sealed from room air, and sampling mechanism comes into play; *B*, mouthpiece, with tube leading to suction tube; *C*, suction tube, communicating consecutively with rubber bulb and evacuated sample barrel during one complete rotation of *A*; *D*, clasp, rotated by tap *A*, which holds tap of suction tube; *E*, rubber bulb (Politzer no. 288) of 180 cc capacity, which evacuates oral cavity and washes out *B* and *C* with alveolar air; *F*, evacuated glass sampling barrel; *G*, mercury reservoir for evacuating sampling tube; *H*, room air aperture with latex indicator, onto which is attached slide valve for rapid switch to spirometer or CO_2 cylinder; *I*, spirometer opening on slide valve; *J*, CO_2 intake opening on slide valve.

Received for publication August 23, 1955.

[1] This work was supported by a grant from the American Heart Association, and was carried out in the Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

TABLE 1

Subj.	Pulse *per min.*	Respir. *per min.*	Arterial Blood CO_2 *mm Hg*	Arterial Alv. Air CO_2 *mm Hg*	Venous Alv. Air CO_2 *mm Hg*	CO_2 Output *cc/min.*	A-V Diff. *vol. %*	Cardiac Output *l/min.*
				Basal Subjects				
1	58	9	35.0	34.07	42.70	190.3	4.1	4.64
2	64	16	39.1	39.20	45.40	246.0	4.2	5.86
3		20		42.67	57.39	216.0	5.0	4.32
4		24	34.5	35.01	45.58	250.0	4.7	5.32
5	64	12		36.24	42.49	192.2	3.2	6.08
6	50	16	44.4	45.30	57.30	221.7	4.8	4.62
7		16	39.7	38.49	46.29	167.2	3.6	4.62
*8**	60	10	42.6	(37.0)	48.70	220.5	5.0	4.41
9		8	40.9	41.49	52.61	215.8	4.5	4.80
				Moderate Exercise				
1		8	39.7	40.15	55.31	930.4	6.2	15.00
2		12	37.0	38.9	49.14	843.0	5.5	15.20
4		20		33.22	42.55	742.0	6.9	10.75
6		16	43.6	44.08	56.16	686.3	4.5	14.04
7†			40.3	(36.62)	56.73	1024.7	7.0	14.60
8		20		40.35	61.17	934.9	8.5	10.90
9		19	35.7	41.27	62.49	1229.9	9.4	13.40

* In *subject 8* arterial blood value was used for cardiac output calculation as alveolar sample was taken late owing to hyperventilation caused by disturbance of arterial catheter. In the other cases blood values were used merely as a check. † Late sampling in *subject 7* caused dilution by next inspiration, and blood value is used for calculation.

subject to breathe one normal breath of the mixture. Immediately at the end of this breath an alveolar air sample was taken, and this procedure was repeated three times. These latter samples represented the venous values, a mean of the three being taken. Finally, the spirometer was utilized to collect the expired air, and a sample of this was analyzed in order to estimate the CO_2 output. The whole procedure on each subject, excluding the period of settling down, was completed in under ½ hour, during which time the subject was on the bed in an attitude of complete rest. The above procedures were then repeated with the subject seated on a bicycle ergometer doing approximately 60 rpm and carrying a load of 5 pounds, after allowing 5 minutes for the subject to settle down to a steady rate while breathing through the apparatus.

The results are given in table 1.

DISCUSSION

The direct method of studying the pulmonary circulation presents attractive features; however, although cardiac catheterization has proved to be a fairly simple procedure and is used for obtaining samples of the mixed venous blood, it has limited application and in order to determine the composition of the blood entering and leaving the lungs under varying conditions of rest and exercise an indirect method of obtaining the information is desirable. At the same time it must be remembered that the pulmonary vascular tree is like other parts of the circulatory system in having a larger capillary bed than is required for ordinary demands (4). Only a proportion of the capillaries carries blood at one time, which implies a reserve for aerating increases in the cardiac output, susceptible of reduction by disease.

Early attempts at carbon dioxide equilibration have been criticized because: *1*) recirculation of inspired CO_2 confused the readings; *2*) voluntary cooperation implied, *a*) previous training, *b*) alteration in the values to be measured on account of the change in the circulation rate, *c*) delay, leading to recirculation problems; *3*) inadequate initial 'boost' with CO_2 left the pulmonary vascular bed partly at arterial tension; *4*) respiratory and instrumental dead-space contamination during inspiration and sampling led to dilution. The present method enables the investigator to procure samples of gas from the lungs under conditions which render the cooperation of the patient unnecessary. Samples have been compared with blood and also Haldane-Priestley values. The procedure can be adapted to the examination, not only of the 'arterial' alveolar

air but of the 'venous' alveolar air, and hence the composition of the mixed venous blood entering the lungs. Although 15% CO_2 in oxygen was used in the experiments reported here, the interesting observation was made that as much as 25% CO_2 brought the alveolar air to a CO_2 tension which was reproducible and which could be taken to represent that of the mixed venous blood. These findings have been discussed (1), and are considerably simplified in figure 2.

It will be noted that the customary use of rubber bags for equilibration purposes is abandoned, the lungs alone being used. This eliminates a source of considerable inaccuracy. It seems fallacious to use a so-called 'lung-bag system' because lungs, physiologically speaking, have no resemblance to bags, which, if used, merely represent an enormous extra dead space (fig. 3).

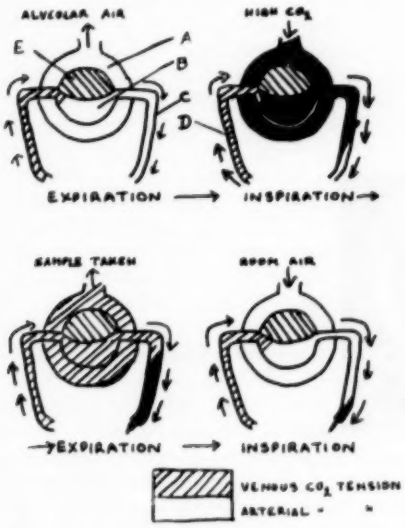

FIG. 2. *A*, alveolar air in lungs. When 15% CO_2 carbon dioxide is inhaled, alveolar air momentarily assumes a CO_2 tension somewhat lower than that of inspired gas but still higher than that of mixed venous blood, *D*, and the vascular bed, *B* (and the alveolar air) assumes the venous tension, the excess CO_2 being carried away by the pulmonary vein, *C*. *E*, 'pulmonary reserve' of unused vessels, which remain quiescent throughout this short procedure (one normal breath) since no effort is required of the patient. The sample is taken by suction from alveolar air, thus obviating dead space and bag contamination, and ensuring exact timing before recirculation of inspired CO_2.

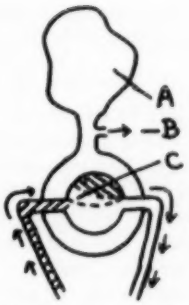

FIG. 3. Objections to conventional 'lung-bag system' for carbon dioxide equilibration. *A*, rubber bag represents enormous additional dead space, composed of CO_2 mixture plus alveolar air, hindered by slow and unequal 'mixing' (in contrast to rapid mixing in the lungs); *B*, sample is diluted with instrumental and respiratory dead-space air since it is collected distal to the mouth, usually from the bag; *C*, variations in CO_2 tension of the pulmonary vascular bed are caused by changes in the right ventricle output due to procedures requiring patient cooperation. This also probably alters the ratios of the blood stream returning from different areas.

It is recognized that good reproducibility does not exclude systematic errors, and it is possible that intracardiac blood samples might help. Even this is in doubt and it may be reasonably claimed that gross systematic error is unlikely. The measurements gave data from which the cardiac output was calculated by means of the Fick Principle. The Haldane dissociation curve was used to determine the CO_2 content of the blood from a knowledge of the partial pressures since information regarding the acid-base buffers, and hemoglobin concentration was not available on each subject. In the absence of simultaneous direct estimations of the cardiac output, observations on the validity of these additional calculations of output are perhaps outside the scope of the present discussion, but they are included as a matter of interest since they appear to be within an acceptable range.

Probably the most important findings are the changes induced by exercise, which suggest that carbon dioxide equilibration methods, which have seen so many disappointed advocates since Haldane *et al.* (6) in 1914 first explored the possibilities of rebreathing, may

yet prove to have the potentialities that the great physiologist felt them to possess.

SUMMARY

The pulmonary blood flow was examined in nine medical students under basal conditions and during the performance of moderate exercise. A new method for determining the carbon dioxide tension of the mixed venous blood entering the lungs was used. This is a carbon dioxide equilibration procedure which does not require cooperation by the patient, and which is completed before coronary recirculation (i.e. within the space of one breath). The significance and application of the findings are briefly discussed.

I am indebted to Dr. Guy H. Fisk, Director of the Department of Physical Medicine and Rehabilitation, Montreal General Hospital, for permission to publish the above report.

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Comparison of the Roughton-Scholander Syringe and Polarographic Methods for Measuring Tension of Oxygen in Plasma and Whole Blood[1]

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IN RECENT years, there has been increasing awareness of need for knowledge of the tension of oxygen in the blood to estimate pulmonary alveolar-arterial oxygen gradients and vascular shunts in normal persons and patients with pulmonary and cardiac disease. The tension of physically dissolved oxygen in the blood is the driving force propelling the oxygen from the plasma into the peripheral tissues. In the balance between oxygen tension and content of the blood, the oxygen tension is proportional to the physically dissolved oxygen, which, according to Henry's Law, occurs in a quantity directly proportionate to the tension in a gas phase with which the blood is in contact.

A method for estimation of oxygen and carbon dioxide tension in the blood was described by Riley *et al.* (1). This method required that a small bubble of alveolar gas be equilibrated with 1 ml of blood at 37°C in each of two Roughton-Scholander syringe analyzers. The gas bubble was then analyzed for carbon dioxide and oxygen, and the blood oxygen and carbon dioxide tensions were calculated in duplicate. At the same time, the tensions of carbon dioxide and oxygen in the gas were determined by a tonometer method, and the accuracy of the bubble method was compared with the results so obtained. In the beginning this method appeared to be the answer to the problem of estimating tension of gases in blood with a simple and easily reproducible technique. The procedure has specific limitations and has been subsequently modified (2, 3) in an effort to improve it.

Analysis of the data reported by Riley *et al.* (1, 2) discloses that the tension of the various gases in the blood does not equal the tension in the gas phase of the equilibrating tonometer when the partial pressure analyses are done with the Roughton-Scholander syringe. A correction factor needs to be added to the oxygen tension value obtained by the Roughton-Scholander syringe analyzer method. Its use is restricted to the physiologic range of oxygen tension. The limitations of the Roughton-Scholander syringe technique have led us to investigate other methods to find one with a greater range of applicability and accuracy.

Berggren (4), in 1942, reported the use of the polarograph for measuring high oxygen tensions in plasma and blood at 0°C. The procedure was investigated by Morgan and Nahas (5) at the Mayo Clinic, and by Wilson, Ebert and Borden (6) at the University of Minnesota. The polarograph has been found to be suitable for measuring oxygen tensions in the blood at all levels.

A number of problems were encountered in the adaptation of the polarograph for measuring the tension of oxygen in the blood at physiologic levels. The first problem encountered and not readily surmounted was the decrease in tension of oxygen in whole blood and plasma during centrifugation to separate the cells from plasma; second, the loss of oxygen by red cell, white cell and other plasma component metabolism, and third, alterations in tension because of the fall in temperature following removal of the blood from the patient or water bath. Recently, however, we have devised a method of centrifuging blood in a container of simple design. The container permits separation of plasma and cells with no change in tensions because of the loss of gas dissolved in the plasma, and facilitates ejection of its contents into a cuvette.

Received for publication August 8, 1955.

[1] This investigation was supported in part by a research grant (PHS Grant No. G-4258) from the National Institutes of Health.

In this report we are presenting the data concerning a method of measuring oxygen tensions in plasma at high and physiologic levels, using the polarograph with a dropping mercury electrode. This method has proved to be accurate over a wide range of oxygen tension. The following is a comparison of data obtained from the Roughton-Scholander syringe and polarograph.

METHOD

The oxygen tension in the plasma was measured directly with the polarograph (fig. 1) at 38°C. Reproducible and quantitative values with this method were obtained from day to day if one worked with a constant drop time and flow of mercury. This was done by removing all foreign matter from the dropping mercury electrode. The tension of oxygen in solution obtained with the polarograph is directly proportional to the diffusion current represented by the following equation (7):

$i = 0.63 \cdot V \cdot F \cdot C \cdot D^{1/2} M^{2/3} \cdot t^{1/6}$, where V = velocity, F = faraday, C = concentration, D = diffusion coefficient, M = flow of Hg/sec., t = drop time.

Gases with different partial pressure of oxygen and carbon dioxide were equilibrated with blood for 30 minutes at 38°C and atmospheric pressure. A 500-ml tonometer (fig. 2) was immersed in a constant temperature water bath and slowly rotated to facilitate equilibration of the blood and gas and to prevent hemolysis because this increased the polarographic reading. The ratio between the quantity of blood and gas equilibrated was 1:25. The exterior of the tonometer was siliconed to prevent cooling by evaporation of water clinging to the sides upon removal from the water bath. Periodically a stopcock on the tonometer was opened to facilitate pressure equilibrium with the atmosphere. Upon establishment of equilibrium between the blood and gas, the tonometer was removed from the water bath, placed in a rack and wrapped with an electric tonometer heating band to maintain the temperature at 38°C. The blood was allowed to drain into the narrow section of the tonometer and removed, precautions having been taken to prevent access of air. An alternate method for removal of the blood without changing the tension of the gases in the tonometer was to admit mercury into a trap through the upper stopcock as the blood was withdrawn (fig. 2). The lower section of the tonometer had a volume of 20 ml so that a relatively small cross-sectional area existed at the gaseous and liquid interphase. The blood was withdrawn into a 5-cc container, as shown in figure 3. This centrifugal container was constructed from a Luer-Lok syringe with finger grips removed. An aluminum alloy plunger was machined with precision so that, when covered with heavy stopcock grease, plasma could not escape between the barrel and plunger of the container during centrifugation. The container was placed in an angle centrifuge in a constant temperature cabinet and spun for 2 minutes at 38°C.

Upon completion of centrifugation, the plasma was ejected over mercury into a 1-cc cuvette with a narrow

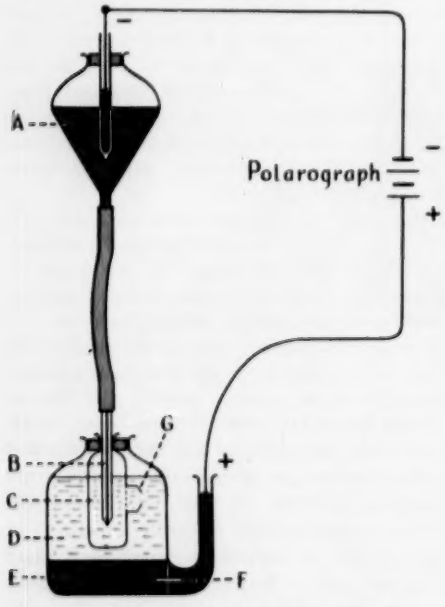

FIG. 1. Schematic diagram of polarograph and dropping mercury electrode. *A*, mercury reservoir; *B*, dropping mercury electrode; *C*, cuvette; *D*, saturated potassium chloride; *E*, mercury pool; *F*, platinum wire bridge; *G*, agar potassium chloride bridge.

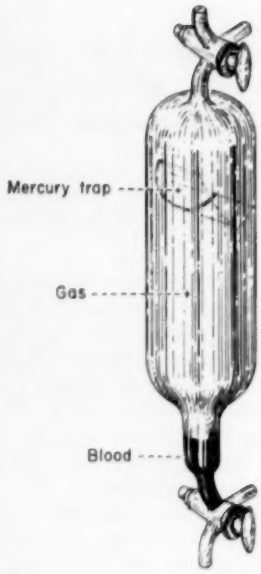

FIG. 2. The 500-ml tonometer used in this study.

neck, 1.5 cm in length, and an internal bore of 1.5 mm which prevented exchange of oxygen between the plasma and the atmosphere (fig. 1). The polarograph was set so that oxygen in the plasma would be reduced at an electromotive force of 0.4 volts. At this voltage the diffusion current of the dissolved oxygen is a linear function of its tension.

The rate of oxygen utilization by whole blood at 38°C was obtained with both the dropping mercury electrode and a platinum microelectrode. Oxygen utilization of the plasma was measured in the same manner. The residual current was obtained with the use of oxygen-free whole blood and plasma.

A factor for converting polarographic readings to oxygen tension was obtained by equilibrating blood and oxygen in the tonometer at different tensions and obtaining polarographic readings (figs. 4 and 5).

In performance of the procedure, it was important that the centrifugal container, the cuvette, the centrifuge, the water bath and the mercury in the dropping electrode reservoir be maintained at 38°C. Duplicate

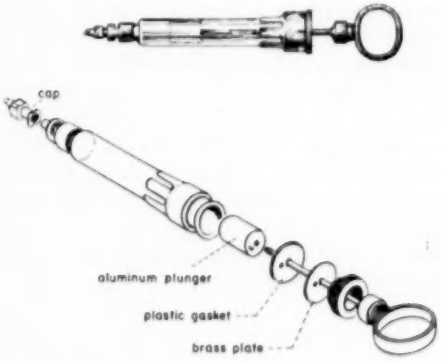

FIG. 3. Syringe modified for rapid centrifugation and ejection of plasma into cuvette.

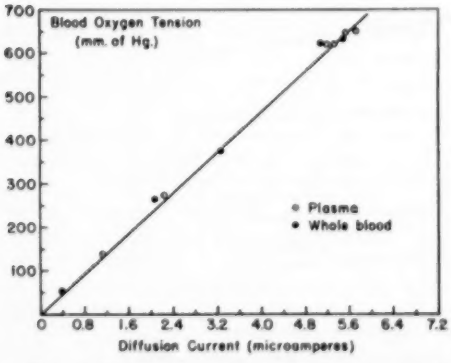

FIG. 4. Relationship between diffusion current in microamperes and tension of oxygen in blood ranging from 50 to 650 mm Hg.

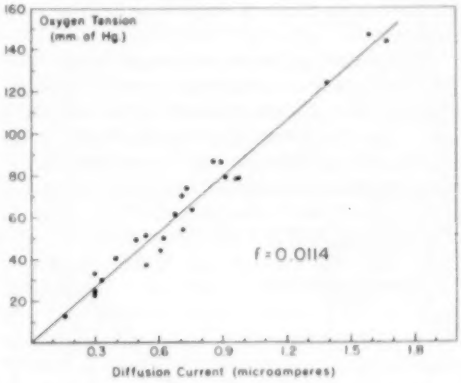

FIG. 5. Relationship of physiologic oxygen tension of blood and polarographic diffusion current in microamperes.

samples of the gas from the tonometer were analyzed by the method of Van Slyke and Sendroy (8). The Roughton-Scholander syringe and polarographic analyses were performed on duplicate aliquots of blood taken from the tonometer.

RESULTS

The comparative data from 21 tonometric samples of blood and gas were used to test the validity of oxygen tension data collected with the Roughton-Scholander syringe and the polarograph.

The linearity of the polarographic reading in microamperes plotted on the X axis and the tension of oxygen in the gas phases in the tonometer on the Y axis are shown in figures 4 and 5. The general equation (9) for the slope of the line representing this relationship is $y = mx + b$. If one substitutes for X the polarographic reading in microamperes, the tension of the oxygen in any sample of blood may be obtained. The constant b is used when the line does not pass through the origin, and m is the slope of the line. The polarographic procedure may be used for measuring the tension of oxygen in blood from 0 to 760 mm Hg at sea level at 38°C. In 21 measurements the corrected mean tension of the oxygen measured with the polarograph was 1 mm Hg greater than that of the Roughton-Scholander syringe (fig. 6). In this study the range of error was greater with the Roughton-Scholander syringe than with the polarographic

method at tensions of oxygen below 100 mm Hg.

The rate of utilization of oxygen by normal whole blood was more than 1 mm Hg/min. (fig. 7). The rate for plasma was 0.1 mm Hg/min. (fig. 7). Whole blood and plasma from which all oxygen has been removed show polarographic curves which remain at a constant level (fig. 7), thus indicating that the decrease in the polarometric curve from minute to minute was because of a fall in oxygen tension. This fall in tension is corrected for during the obtainment of the factor for converting microamperes to oxygen tension because there is no change in the tension of the oxygen in the gas phase of the tonometer which is in equilibrium with the blood at the instant of separation of the whole blood and gas. The Roughton-Scholander syringe method of measuring oxygen tensions in whole blood depends not only on a numerical correction factor for its final value, but the oxygen tension of the blood in the syringe analyzer is increased or decreased when the bubble of gas for equilibration is admitted. While there is no limit to the oxygen tensions which might be measured with the polarograph (6), the usefulness of the Roughton-Scholander syringe is restricted to physiologic levels. The most accurate data obtained by the latter method was at oxygen tension levels within the range of the steepest slope of the oxyhemoglobin dissociation curve. The polarometric method is applicable at the extremes of the oxyhemoglobin dissociation curve where use of the syringe method for measuring tension would be most difficult (fig. 4).

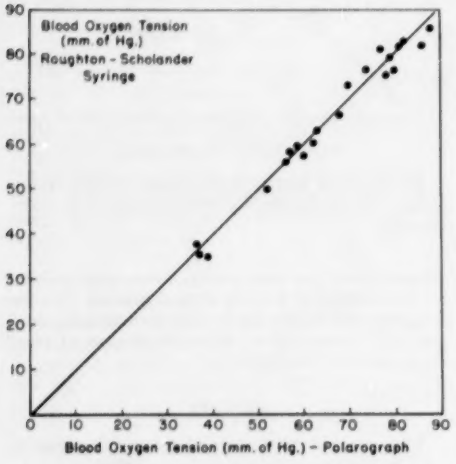

FIG. 6. Comparative physiologic levels of oxygen tension in blood obtained with Roughton-Scholander syringe and polarographic methods.

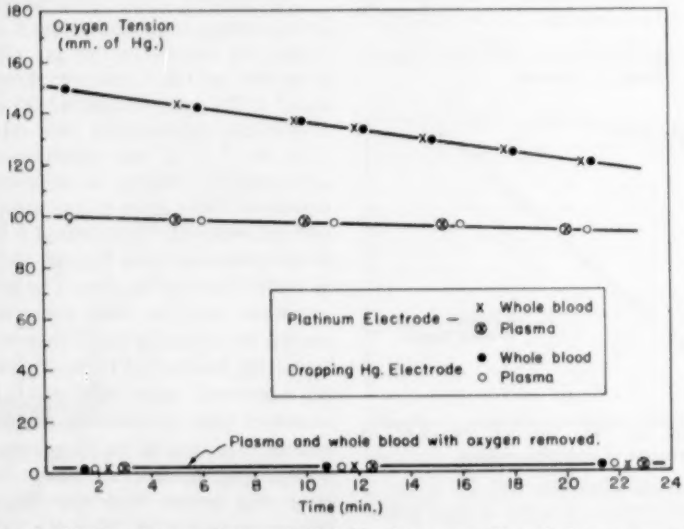

FIG. 7. Rate of utilization of oxygen by normal whole blood at 150 mm Hg and plasma at 100 mm Hg tension. These data were obtained with polarographic dropping mercury and platinum electrodes.

DISCUSSION

Comparison of the blood oxygen tension data collected with the Roughton-Scholander syringe and the polarograph reveals that the physiologic partial pressures of oxygen in the blood measured with the syringe analyzer method is approximately 1 mm Hg lower when a Roughton-Scholander syringe correction factor is used and a bubble of specific composition is employed in the Roughton-Scholander syringe, as Riley has suggested. Beyond the physiologic level of 100 mm Hg, the partial pressure of oxygen can be estimated with the Roughton-Scholander syringe only with great difficulty. Estimating the partial pressure of oxygen and carbon dioxide in whole blood with the Roughton-Scholander syringe technique requires great skill. It is not a simple method. In a recent report, Riley *et al.* (2) discussed the technique thoroughly. The importance of rigidly controlling such factors as preparation of the syringe, use of a standard equilibrating gas, a bubble of standard size, and reducion of the time for equilibration, and the use of a telescope and a vernier scale was emphasized, and that careful consideration of these aspects of the techniques was imperative for the most accurate results. In the hands of the personnel of this laboratory, the results were the same as those of Riley and co-workers. We found it necessary to use a correction factor for the oxygen tension if it were to equal that of the tension of the partial pressure of the oxygen in the gas of the tonometer. It is not possible to obtain accurate results without the use of a correction factor and a bubble of gas of specific size and composition. Neither is it feasible to state that a correction factor is not needed when the tonometer gas with which the blood is equilibrated is used for the gas bubble in the syringe, because in instances where one is determining the tensions of oxygen in an unknown sample, such as in the blood of a patient, tonometer gas may not be suitable. Therefore, to obtain an accurate correction factor, one must use a standard equilibrating gas composed of oxygen, carbon dioxide and nitrogen. It is not imperative that one employ a bubble composed of exactly 11.65% oxygen, 6.10% carbon dioxide and 82.25% nitrogen. Acceptable results may be obtained by using a syringe bubble composed of gases that contain slightly more or less oxygen and carbon dioxide than one of the specific compositions outlined above, if the same gas is employed for establishing the correction factors and running the unknown sample. If a standard gas of slightly different composition from that reported is used, the correction factors will be different at a given oxygen tension level.

Estimation of the partial pressures of oxygen in the blood with the polarograph and dropping mercury electrode requires that the cells be separated from the plasma without hemolysis. This is accomplished by rinsing the centrifugal containers, tonometers, syringes and needles with normal saline prior to the procedure. It was demonstrated by Berggren (4) and Wilson *et al.* (6) that hemolysis caused the polarographic readings to be too great.

The effect of the variations of temperature on the oxygen diffusion current has been found to be significant. The diffusion current diminishes with the fall in temperature. This phenomenon was studied extensively by Berggren (4) and his co-workers. They found that the decrease in the diffusion current was considerably stronger in blood or hemoglobin solutions than with plasma. With cooling of blood, there is increased affinity between hemoglobin and oxygen. For this reason it is necessary to measure the oxygen tension of the blood at 38°C.

Collection of small particles of fibrin and other proteins in the lumen of the tip of the capillary from which the mercury is flowing will alter the drop time and quantity of mercury flowing per second. If the drop time and the quantity of mercury changes, the calibration factor will have a new value. Since it is necessary to have absolute constancy of the drop time and the quantity of mercury flowing from day to day, it was found necessary to check the calibration factor with at least one tonometric equilibration of blood and gas each day and plot a calibration curve. If one immerses the tip of the dropping mercury electrode in 30% nitric acid, rinses with detergent and distilled water, the drop time and the quantity of mercury flowing through the electrode may be constant for several months. The flow of mercury through the electrode should be continuous to prevent aspiration of foreign particles into the lumen of the glass electrode. It is essential to use triple-distilled mercury free of all impurities.

It has been found that closed cuvette systems which lead to an increase of pressure as

the mercury falls from the dropping electrode, alter the calibration factor (7). Thus, a cuvette was devised in which the pressure remained at atmospheric level during the procedure. Periodically, the KCl agar bridge in the cuvette must be replaced. The components of the agar are to be found in Kolthoff and Lingaine's (7) extensive work on the polarograph. During periods the cuvette is not in use, it must be immersed in saturated potassium chloride solution to prevent dehydration of the KCl agar bridge.

The red and white cells of the blood utilize oxygen at body temperature. The decrease in oxygen tension is a small but variable quantity, and is approximately 1 mm Hg/min. at body temperature when the tension of the oxygen is 150 mm Hg. At physiologic levels, this decrease is oxygen tension will be the negligible quantity of 1 mm Hg/hr. This will vary with the hemoglobin and white cell content of the blood. From the instant the blood was removed from the tonometer to the time the dropping mercury electrode was inserted into the cuvette, the period of time was 3 minutes; thus, the polarographic readings are corrected by using the tension of the oxygen in the gas phase of the tonometer at zero time. The blood was centrifuged in a small angle centrifuge at approximately 5000 rpm at 38°C for 2 minutes. One minute was required to stop the centrifuge and eject the plasma from the centrifugal syringe into the cuvette. The cuvette was filled with mercury at 38°C prior to the procedure.

The container in which the blood was centrifuged was filled with normal saline and heated to 38°C. This provides a constant temperature in the container during manipulations prior to extraction of a sample of blood from a patient or tonometer, and is a means of heating and rinsing the tonometer stopcock with saline to remove water from the side arm and bore of the stopcock to prevent hemolysis.

SUMMARY

The polarograph may be used to measure the partial pressure of oxygen from 0 to 760 mm Hg in blood after separation of the cells from the plasma by centrifugation. The diffusion current of oxygen in plasma is directly proportional to the tension of the oxygen in solution. The rate of oxygen consumption at 150 mm Hg tension in normal whole blood is enough to cause a fall of the tension level of approximately 1 mm Hg/min. The rate of fall of oxygen tension in plasma without cells is 0.1 mm Hg/min. at the 100-mm Hg level, but may vary from sample to sample. In the instance of whole blood, the rate of fall of the oxygen tension will depend on its initial level, the temperature and number and state of the leucocytes and erythrocytes. In the physiologic range of oxygen tension, the slope of the oxyhemoglobin dissociation curve is great enough so that the change in tension because of oxygen consumption by whole blood is a lower order of magnitude; therefore, the Roughton-Scholander syringe data need not be corrected for this deficiency. The polarographic factor for converting microamperes to mm Hg tension of oxygen corrects the blood for the fall in tension from the time the blood is withdrawn from a patient or a tonometer. The oxygen tension in the blood obtained by the Roughton-Scholander syringe after the addition of the correction factor, as suggested by Riley, is 1 mm Hg lower than that of the polarograph. If the correction factor is not applied to the partial pressure of the oxygen obtained by the Roughton-Scholander syringe, the results obtained by this method will not be equal to partial pressure of the gases in the tonometer. It is not possible to estimate correct partial pressures of oxygen in the blood with the Roughton-Scholander syringe without addition of a correction factor and the use of a bubble of gas of specific composition and size having a partial pressure of oxygen within the range of 85–100 mm Hg and a carbon dioxide tension of 40 mm Hg.

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Effect of Skin Diving on Lung Volumes

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OBSERVATIONS in the laboratory as well as in practice suggested that in the course of their duties the instructors at the Escape Training Tank underwent some changes in their pulmonary capacity. For example, it was found that the instructors could hold their breath much longer than the average individual.

The instructors at the Escape Training Tank are engaged almost daily in 'skin diving,' that is diving without the use of any special equipment. The instructors take a breath of air at the surface, descend in the tank to depths as great as 100 feet, and return to the surface while holding their breath. They also make 'free ascents,' which means that while in an air lock at 25, 50 or 100 feet under water they take a deep breath, step out of the lock into the water and float to the surface, exhaling the expanding air in their lungs as they rise. The free ascent is performed by the instructors as they guide the Submarine School students through a series of escapes from the air locks. The student uses the Submarine Escape Appliance, a self-contained breathing apparatus, during his ascent to the surface. If the student has become thoroughly familiar with this ascent technique, he is given the opportunity to perform free ascents himself.

Skin diving involves the potential hazard of a squeeze. During free escape the danger exists of overdistention of the lungs, with possible rupture (1, 2). Kinsey (3) published a report on four cases of air embolism as a result of submarine escape training. Even in proper execution of skin diving and free ascent there is a certain amount of stress imposed on the lungs; this is readily demonstrated by considering the pressure-volume relationships in the lungs.

The pressure at 100 feet is equal to four atmospheres absolute. Therefore, a volume of air inhaled at the surface will be compressed to one-fourth of its original volume at that depth; conversely, a lungful of air inhaled in the air lock at 100 feet will expand to four times its volume when reaching the surface. (While stationed in the 100-ft. lock, during escape training, the instructors may be exposed to 4 atmospheres absolute pressure for periods up to 20 min.)

We are interested in knowing whether the instructors do adapt to the stress of skin diving by increasing their lung volumes. This would explain their observations that, during their tour of duty, they learn to hold their breath longer and breathe differently.

Received for publication August 1, 1955.

MATERIAL AND METHODS

Three groups of subjects were studied. *Group A* consisted of 16 instructors at the Escape Training Tank who had carried out 'in-the-water' instruction for an average period of 1½ years. *Group B* was composed of 16 adult male personnel from the Medical Research Laboratory. After it was found that the lung volumes of the tank instructors were markedly larger than those of the laboratory personnel, a longitudinal study was performed on a group of 20 tank instructors. Pulmonary capacities were measured within the first 2 months of their tours of duty at the Escape Training Tank and after approximately 1 year. In a group of eight subjects, additional determinations of the lung volumes were made with the subjects standing on a ladder up to the neck in water. Under these conditions the intrapulmonic pressure in reference to the ambient water pressure was measured at −20 mm Hg.

Total lung capacity and its subdivisions were studied and the measurements taken as follows: *a)* vital capacity, as measured from the maximum expiration following a maximum inspiration, was determined using the Collins Vitalometer, the highest value of three successive trials recorded. *b)* Inspiratory and expiratory reserve and tidal volume were measured using the Collins Respirometer. The soda-lime canister was removed to minimize the resistance to breathing. The respirometer was flushed with room air after each measurement to prevent carbon dioxide from accumulating in the closed system. *c)* Residual volume was determined by the Rahn, Fenn and Otis modification of the Lundsgaard-Van Slyke method (4). The alveolar

air samples were analyzed for carbon dioxide and oxygen content in a Scholander respiratory gas analyzer (5). All values reported were calculated to body temperature, ambient pressure and saturated with water vapor (BTPS). The determinations were carried out with the subjects in a standing position in order to make a comparison of the various pulmonary subdivisions out of the water and in the water.

RESULTS

Vital Capacities of Laboratory Personnel and Tank Instructors. Vital capacities of the two groups measured with the Vitalometer were found significantly different (table 1). The tank instructors showed greater vital capacity. A comparison of predicted and measured vital capacities is given in table 1. Predicted values were obtained by using the West formula, which is based on body surface area (6).

The mean age for each of the two groups was not found to be significantly different, the laboratory personnel averaging 30.4 years and the instructors 30.3 years. The results indicate that the vital capacities of the laboratory personnel fall in the range of predicted values, while the vital capacities of the instructors are larger. For the group of instructors the mean measured vital capacity is significantly higher (5.68 liters) than the mean predicted value (4.93 liters) based on body surface area. The mean measured vital capacity of the laboratory personnel (4.86 liters) is not significantly different from the value predicted (4.82 liters). These values represented as percentage deviation of the measured from the predicted vital capacities showed the instructors to be +14.56% higher than predicted, while the laboratory personnel were −0.56% but not significantly lower.

Lung Volumes of Tank Instructors and Laboratory Personnel (Table 2). Measurement of lung volumes of the two groups revealed that the tank instructors had significantly larger total lung capacities (6.990 liters) than the laboratory personnel (6.120 liters). Of the subdivisions of the total lung capacity, vital capacity, tidal volume and inspiratory reserve are larger in tank instructors than in the laboratory personnel.

Longitudinal Study. Table 3 shows the results of measurements of the pulmonary volume at the beginning of duty and after approximately 1 year of work in the water. The volume of all subdivisions of the lungs increases except that of the residual volume, which decreases.

The statistical evaluation of these data given in table 3 reveals changes that are significant

TABLE 1. COMPARISON OF VITAL CAPACITIES OF LABORATORY PERSONNEL AND TANK INSTRUCTORS

	Predicted Vital Capacity Based on Body Surface Area	Measured Vital Capacity	Deviation of Measured From Predicted Vital Capacity*
	Liters (BTPS)	*Liters (BTPS)*	*%*
		Laboratory Personnel	
1	5.25	3.89	−26.0
2	4.40	4.02	−8.6
3	5.02	5.00	−0.4
4	4.87	3.80	−22.0
5	4.60	4.47	−3.0
6	4.70	5.82	+23.9
7	4.92	5.47	+11.0
8	5.02	4.52	−10.0
9	4.63	4.67	+5.5
10	5.12	4.47	−12.7
11	4.65	4.63	−0.4
12	4.50	5.11	+13.5
13	5.06	4.88	−3.6
14	5.05	5.69	+12.7
15	4.55	4.63	+1.8
16	5.50	6.02	+9.4
Mean	4.86	4.82	−0.56
S.D.	.38	.63	12.91
		Tank Instructors	
1	4.78	4.43	−7.3
2	4.38	5.00	+14.1
3	4.68	5.33	+14.1
4	4.80	6.33	+31.9
5	5.10	6.25	+22.5
6	4.88	5.59	+14.5
7	4.83	5.56	+15.1
8	5.10	5.38	+5.5
9	4.65	4.90	+5.4
10	5.10	5.89	+15.0
11	4.70	5.60	+9.0
12	5.00	6.60	+32.0
13	5.00	6.27	+25.0
14	5.38	6.00	+12.0
15	4.75	5.24	+10.3
16	5.70	6.49	+13.8
Mean	4.93	5.68†	+14.56*
S.D.	.25	.59	9.60
P	>.1	<.001	<.001

* Predicted vital capacity based on body surface—100%.

† Difference statistically significant to the 1% level or less.

in: *a*) tidal volume, which increases during the 1-year period from 1.10 liters to 1.25 liters; *b*) inspiratory reserve, which increases from 2.65 liters to 2.80 liters; and, as a result of the above two changes; *c*) vital capacity increased from 5.17 liters to 5.63 liters; *d*) total lung capacity increased from 6.89 liters to 7.27 liters, a change significant to the 0.1% level of confidence. Expiratory reserve, residual volume, and consequently, functional residual capacity are not found to have changed appreciably.

TABLE 2. PULMONARY CAPACITIES OF LABORATORY PERSONNEL AND TANK INSTRUCTORS

	Laboratory Personnel		Tank Instructors
	cc (BTPS)		*cc (BTPS)*
Tidal volume			
Mean	759.7		1018
S.D.	247.4		501.4
Number	16		16
P		>.1	
Inspiratory reserve			
Mean	2489*		3057.2
S.D.	491.7		698.1
Number	16		16
P		<.02	
Expiratory reserve			
Mean	1405.4		1316.9
S.D.	523.8		482.3
Number	16		16
P		>.1	
Vital capacity			
Mean	4654.7*		5397.4
S.D.	656.1		555.7
Number	16		16
P		<.01	
Residual volume			
Mean	1465.8		1592.7
S.D.	322.6		301.4
Number	16		16
P		>.1	
Functional residual capacity			
Mean	2871.4		3073.4
S.D.	585.8		764.8
Number	16		16
P		>.1	
Total lung capacity			
Mean	6120.2*		6990.1
S.D.	729.3		698.1
Number	16		16
P		<.01	

* Difference statistically significant to the 2% level or less.

TABLE 3. EFFECT OF SKIN DIVING ON LUNG VOLUMES

	I Initial Measurement		II After One Year Duty at Escape Training Tank
	cc (BTPS)		*cc (BTPS)*
Tidal volume			
Mean	1101		1255*
S.D.	435		481
Number	20		20
P		<.05	
Inspiratory reserve			
Mean	2652		2803*
S.D.	374		432
Number	20		20
P		<.05	
Expiratory reserve			
Mean	1424		1569
S.D.	490		530
Number	20		20
P		>.1	
Vital capacity			
Mean	5175		5629*
S.D.	622		760
Number	20		20
P		<.001	
Residual capacity			
Mean	1768		1655
S.D.	499		372
Number	20		20
P		>.1	
Functional residual capacity			
Mean	3192		3224
S.D.	866		700
Number	20		20
P		>.1	
Total capacity			
Mean	6893		7274*
S.D.	971		880
Number	20		20
P		<.001	

* Difference statistically significant at the 5% level or less.

Table 4 shows the results of measurements of lung volumes made on a small group of eight subjects in the water over a period of 1 year. It can be seen that the same lung volumes which were found to have increased after a period of 1 year in determinations made out of the water also increased when measured in the water, i.e. under a negative

TABLE 4. COMPARISON OF MEASUREMENT OF PULMONARY CAPACITY OF DIVING INSTRUCTORS IN AND OUT OF THE WATER

	Out of Water		In the Water	
	Initial	After 1 yr. of duty at the Escape Training Tank	Initial	After 1 yr. of duty at the Escape Training Tank
	cc (*BTPS*)	cc (*BTPS*)	cc (*BTPS*)	cc (*BTPS*)
Tidal volume				
Mean	1146	1390	1353	1370
S.D.	292	510	444	228
Number	8	8	8	8
P	> .1		> .1	
Inspiratory reserve				
Mean	2892	2930	3326	3557
S.D.	219	382	560	715
Number	8	8	8	8
P	> .1		> .1	
Expiratory reserve				
Mean	1424	1701	736	816
S.D.	450	554	329	509
Number	8	8	8	8
P	> .1		> .1	
Vital capacity				
Mean	5454	6021*	5408	5691
S.D.	580	848	637	724
Number	8	8	8	8
P	< .05		> .1	
Residual volume				
Mean	1918	1621	2022	1962
S.D.	570	287	616	606
Number	8	8	8	8
P	> .1		> .1	
Functional residual capacity				
Mean	3342	3322	2646	2780
S.D.	854	522	641	944
Number	8	8	8	8
P	> .1		> .1	
Total lung capacity				
Mean	7373	7643	7429	7652
S.D.	961	888	1105	1168
Number	8	8	8	8
P	> .1		> .1	

* Difference statistically significant at the 5% level.

intrapulmonic pressure of −20 mm Hg. Because of the small number of subjects, differences do not become statistically significant, but reflect the same changes observed in examinations carried out in a larger group of subjects out of the water.

DISCUSSION

The larger lung volume of the tank instructors as compared with those of a group of laboratory personnel representing average individuals could be produced by *a*) natural selection and *b*) adaptation. Findings showing the measured vital capacity of tank instructors 14.5% higher than the vital capacity predicted on the basis of body surface area pointed in the direction of an adaptation. The longitudinal study definitely demonstrated that an adaptation takes place and that the tank instructors increase their lung volumes (total capacity, inspiratory reserve, tidal volume, vital capacity) during their tour of duty.

This does not exclude a certain kind of natural selection in the case of very efficient skin divers among the group of tank instructors. They have large lung capacities to start with. But the important finding is that all the tank instructors increase their lung capacity during their tour of duty. The increase in inspiratory reserve and tidal volume is not achieved by a corresponding decrease in other pulmonary volume. Expiratory reserve remains approximately the same. Although residual volume decreases somewhat, the total lung capacity increases significantly.

As to the cause of these adaptive changes in lung volumes, we might examine the working conditions of the tank instructors a little more closely. Prior to diving, they take a deep breath in a position in which they are submerged up to the neck in the water. Under these conditions, the hydrostatic pressure on the chest was measured to be equivalent to a negative intrapulmonary pressure of −20 mm Hg. This finding is in agreement with data given by Rahn *et al.* (7) and Fenn (8). Rahn *et al.* (7) demonstrated that at −20 mm Hg the inspiratory reserve and the tidal volume are markedly increased, while the expiratory reserve is decreased. Therefore the influence of the position in the water helps greatly to overcome the 'stress' of skin diving, which involves maximal inspiration in an effort to hold the breath as long as possible.

The changes in lung volumes produced by the position in the water are similar to those observed during adaptation of the instructor, the difference being that the latter are actual and permanent changes resulting in an increased total lung capacity, while the former are only compensatory changes within the unaltered total lung capacity. Increases in inspiratory reserve, tidal volume, vital capacity and total capacity facilitate maximal

inspiration and increase the breath-holding time. The 'stress' in skin diving definitely is on the inspiratory side of the respiratory cycle, and the adaptation shows a response to this stress.

The observed changes in lung volumes allow the skin diver to reach a lower depth. Whether a skin diver can reach 100 feet or 120 feet without a squeeze depends on the relation of his total lung capacity to his residual air. If, for example, the lung volume of a diver at the surface is 6000 cc and his residual air 1500 cc, he should be able to reach 100 feet (equivalent to 4 atmospheres) because his lung volume would be compressed to one-fourth, or 1500 cc. At 132 feet (5 atmospheres) the lung volume would be compressed to 1250 cc, which is less than his residual air, and lung tissues would be inverted into the larger passages and blood would be squeezed into the bronchi. It is evident that by increasing the total lung capacity or decreasing the residual air the diver can increase the depth to which he is able to go. This is exactly the adaptation which takes place in the instructors. An increase in total lung capacity has been demonstrated; a slight decrease in the residual air is suggested by the measurements. The adaptive changes found in lung volumes of tank instructors bear a relationship to their respiratory pattern and tolerance to increased carbon dioxide tensions, which has been discussed by Schaefer (9).

SUMMARY

Vital capacity, measured with the Collins Vitalometer in a group of laboratory personnel and a group of Escape Training Tank instructors, was found significantly larger in the tank instructors. When the measured vital capacity for each of the two groups was compared with vital capacity values predicted on the basis of body surface area (West formula), it was found that the measured values of the tank instructors were 14.6% higher than predicted, while those of the laboratory personnel were 0.56% lower than predicted. Measurements of the subdivisions of the lung volumes showed that the tank instructors had significantly larger total capacity, vital capacity and inspiratory reserve. A longitudinal study of the lung volumes of instructors carried out for a period of 1 year following their assignment to the Escape Training Tank exhibited a significant increase in total lung capacity, vital capacity, inspiratory reserve and tidal volume. Additional measurements of lung volumes made in a group of tank instructors standing on a ladder up to the neck in water reflected changes in the same direction as observed in determinations made out of the water.

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Oxygen Toxicity Studies in Underwater Swimming

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HUMAN tolerance for breathing oxygen at high pressures has been found to be impaired during work by Yarbrough *et al.* (1), Donald (2), Bornstein and Stroink (3), Behnke (4) and Schaefer *et al.* (5). Some of the previous reports note similar effects when men are under water (1, 2, 5). Practical use of oxygen breathing by underwater swimmers has been considered to be limited to 30 feet (1), or 25 feet (2). These narrow limitations illustrate the recognized danger of oxygen breathing while under water, and were the background for the writer's effort to find signs which might constitute a warning of the approach of acute symptoms.

Two well established cardiovascular findings during exposure to increased percentage of oxygen at one or more atmospheres provided a promising lead for further investigation: *a*) the bradycardia observed by Behnke and other workers (3, 4, 6–11), and reported by Bean and Rottschafer (12) to be of vagal origin; and *b*) the increase noted in pulse rate and blood pressure if symptoms of oxygen toxicity develop in man (1, 3, 4, 6–8). The stimulation of the sympathetic division of the autonomic systems directly accompanying symptoms follows a transitory stimulation of the parasympathetic system, as indicated by the bradycardia. In the work of Donald, degree and onset of pulse rate decrease did not show any fixed relation to symptoms (2). We were interested in finding a sign between these two phases which could be used as a warning, and therefore studied the pulse rate changes at the beginning and end of rest periods during underwater swimming.

In this paper, the term sympathetic activity has been used to describe pulse rate increase associated with symptoms of oxygen toxicity, following the example of other investigators (8). In contrast, the bradycardia and fixation of pulse rate, considered to be of vagal origin, have been referred to as parasympathetic activity.

Received for publication August 1, 1955.

METHOD

Twelve men (trained underwater swimmers) swam in the 100-foot lock of the Submarine Escape Training Tank and in the Submarine Base swimming pool, wearing either the Lambertsen or the Browne underwater swimming units, swim fins and mitts. These underwater swimming units have a rebreathing system with a CO_2 absorption canister and an oxygen supply from an oxygen cylinder containing 97–99% oxygen. The escape tank was flooded to a depth of about 4 feet, with air pressure in the lock equivalent to depths of 20, 30 and 40 feet. The water temperature in the tank was constant at about 90° F. The swim circle was about 36 feet in circumference, and the average rate of swimming 0.98 miles per hour. The subjects swam continuously at this rate, with rest periods as shown in the following schedule:

Swimming	Rest Period
1st–15th min.	15th–17th min.
17th–32nd min.	32nd–36th min.
36th–51st min.	51st–57th min.
57th–72nd min.	72nd–78th min.
78th–93rd min.	End point

In swims at the swimming pool, which were carried out at 2 feet below surface, the same schedule was maintained. The latter differed from the swims in the tank only inasmuch as the swimming circle was wider and the water temperature lower (80° F).

Rest periods were found to be physically necessary during the course of experiments testing exhaustion end points at depths of 30 and 40 feet. They also gave an opportunity *a*) to take gas samples from the bags, and *b*) to measure pulse rate and respiratory rate in the beginning and at the end (counting for 15 seconds).

For practical purposes, the pulse rates measured during the first 15 seconds of the rest periods were considered as exercise values, since it had been shown by Cotton and Dill (13) that in 10 seconds immediately after strenuous exercise the heart rate decreases on an average of about 1 beat per minute only. Lythgoe and Pereira's curves showing the retardation of the pulse rate after a period of exertion (14) indicate a fall of 4–5 beats per minute at 20 seconds post exercise. For practical purposes of comparison, the pulse rates taken at the end of the rest periods in this study (duration: 2, 4 and 6 min.) were considered as resting values, on the basis that the principal drop of the post-exercise values occurs within 2 minutes as reported by Johnson and Schneider and their associates (15, 16), and the time

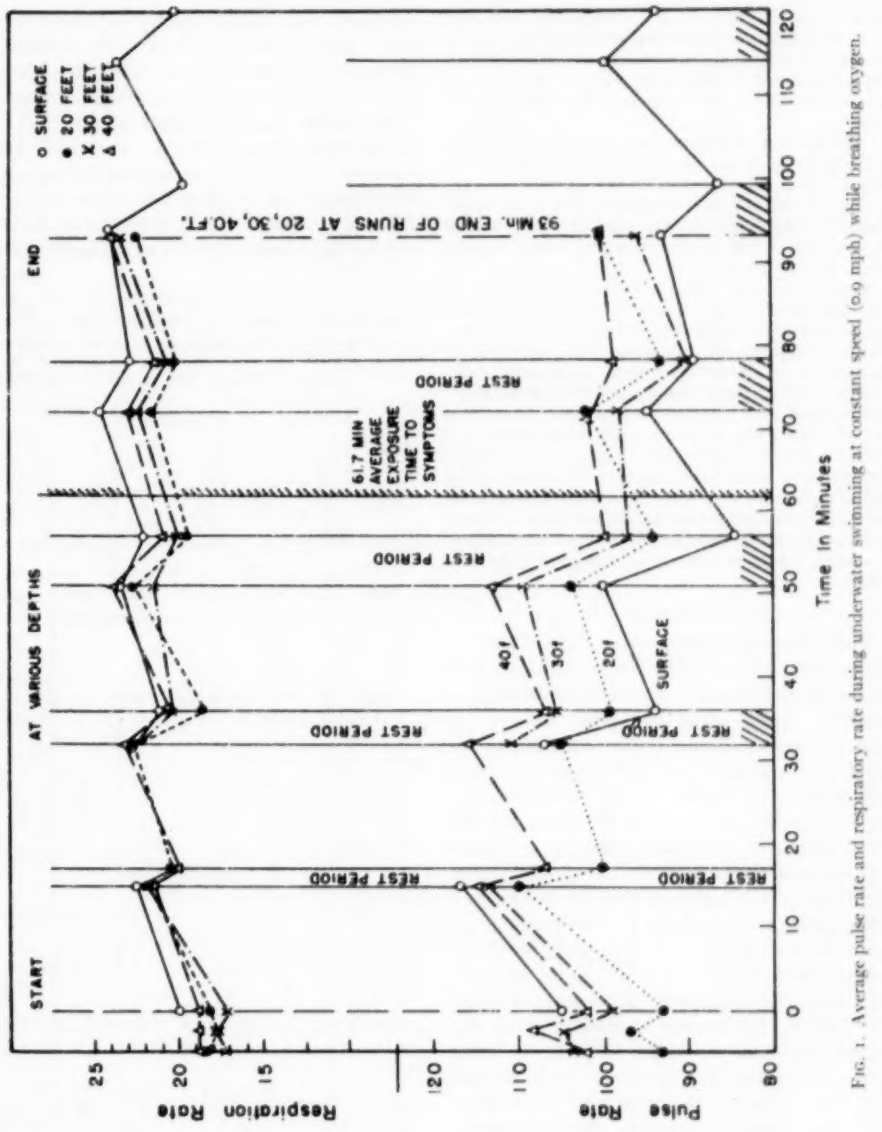


FIG. 1. Average pulse rate and respiratory rate during underwater swimming at constant speed (0.9 mph) while breathing oxygen.

required for the pulse rate to return to normal after moderate exercise is short in trained subjects such as these.

The Lambertsen and Browne underwater swimming units have a rebreathing system with a carbon dioxide absorption canister and an automatic oxygen supply via a demand valve from an oxygen cylinder. Oxygen consumption of the swimmers can be measured via the pressure drop of the oxygen cylinders. The volume of the oxygen cylinders used was measured at 1310, 1397 and 1504 cubic centimeters, by filling the cylinders with water. Calibration of the oxygen cylinders used in these experiments was carried out after the completion of the experiments at 90° F room temperature (90° F was the average water temperature at which the experiments were performed at the tank). The rate of oxygen delivered by the cylinders per 100 psi drop was measured with a wet gas meter. Average delivery rate of

oxygen from the cylinders at a temperature of 90° was used for the calculations of oxygen consumption. The percentage of oxygen contained in the cylinders had been determined in each case, being in the range of 97–99%. All values were corrected to standard pressure, dry.

This method of measurement of oxygen consumption was similar to one used by others in studies at San Diego, and at the Experimental Diving Unit in Washington (17, 18), the differences being that in the latter studies *a*) the oxygen supply cylinders were smaller (500 cc) and *b*) the pressure drop was measured more often, once each lap.

Gas samples were taken from the breathing bag of the underwater swimming units at the beginning and end of the experiment as well as during each rest period. The breathing bag was equipped for gas sampling by installing a pneumatic valve stem, on to which was fitted a 4-inch length of tubing closed by a pinch clamp. Gas samples were collected in glass tonometers over mercury rinsing the sample twice after flushing the first few cubic centimeters from the tubing. CO_2 and oxygen concentration were determined by the Van Slyke method, the majority of the CO_2 determinations being checked by the Haldane method. Results were reported in detail elsewhere (5) and are summarized in this paper in relation to the occurrence of symptoms of oxygen toxicity.

RESULTS

Pulse Rate and Respiratory Rate. Figure 1 shows the average pulse rate and respiratory rate obtained during underwater swimming at constant speed while breathing oxygen at various depths. If attention is given to the pulse rate measured at the beginning of the rest periods, one notes *1*): that there is a systematic downward trend of the pulse rate at each depth; *2*) that from the 32nd minute (second rest period) up to the 72nd minute the pulse rate is higher at greater depths (see table 1*A*); *3*) that there is a comparatively large drop in pulse rate after the third rest period, between the 51st and 72nd minutes (or, otherwise expressed, following the third rest period, moderately strenuous work no longer produces the usual increase in pulse rate). The pulse rate becomes more or less fixed. In contrast to the pulse rate, the respiratory rate shows an upward trend at corresponding time intervals. There are no significant differences in respiratory rate at various depths (see table 1*B*).

During the rest periods, pulse rate and respiratory rate fall as expected. One can see that towards the end of the swims the decrease in pulse rate during the recovery becomes smaller, indicating a reduced responsiveness of the pulse rate. This is clearly expressed in the fourth rest period (72nd min.), which seems to correspond to some extent with the average exposure time before symptoms (61.7 min.).

Individual analyses demonstrate that reduced responsiveness of the pulse rate during recovery periods after swimming precedes the development of symptoms in most cases. In figure 2 this phenomenon is shown to develop step by step during a swim at subsurface after

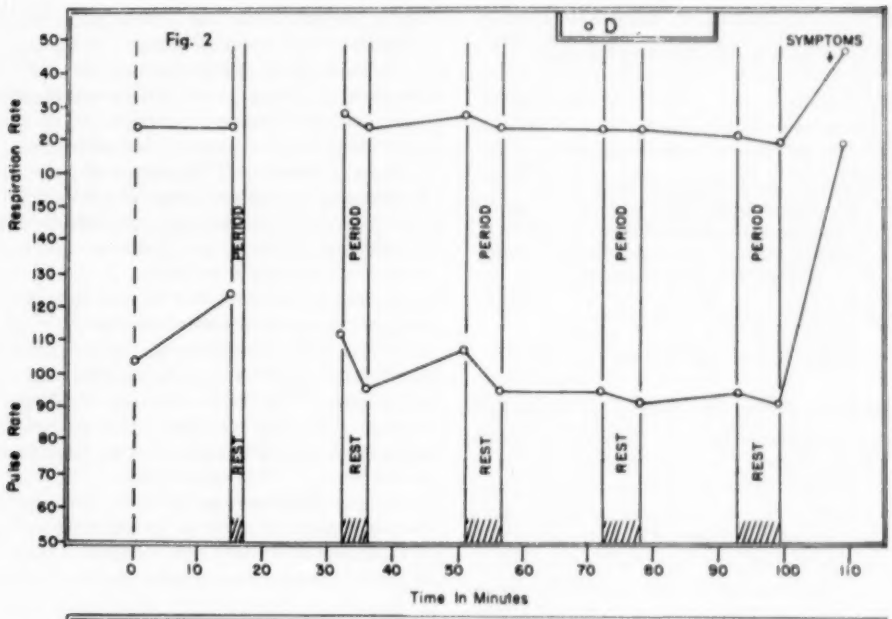
TABLE 1. PULSE AND RESPIRATORY RATES DURING UNDERWATER SWIMMING AT CONSTANT SPEED OF ABOUT .98 MPH WHILE BREATHING O_2 AT VARIOUS DEPTHS

	Start	15 Min.	32 Min.	51 Min.	72 Min.	93 Min.
A. Pulse rates						
Surface						
N	8	8	8	8	7	5
Mean	104.8	117.0	107.0	99.5	95.4	93.6
S.D.	16.4	18.3	15.7	19.9	13.1	25.9
P					.05	
20 ft						
N	11	13	13	8	8	8
Mean	92.9	110.3	105.2	104	102.8	100.8
S.D.	12.7	12.6	13.7	15.4	13.8	17.1
P			*		.1	
30 ft						
N	16	9	12	15	10	10
Mean	99.3	107.9	111.3	109.6	98.8	96.4
S.D.	9.3	16.2	13.4	10.9	11.3	12.3
P					.1	
40 ft						
N	21	19	21	21	13	9
Mean	101.8	114.6	116.4	113.5	120.0	99.6
S.D.	13.3	14.3	13.0	13.9	12.3	14.3
P			.05*		.02	.02
B. Respiratory rates						
Surface						
N	8	8	8	8	8	6
Mean	20.0	22.6	22.5	23.5	24.6	24.0
S.D.	5.0	6.7	4.3	4.8	3.95	4.2
20 ft						
N	12	12	10	9	8	7
Mean	18.4	22.3	22.6	22.7	21.5	22.4
S.D.	1.7	2.8	1.6	4.5	2.18	2.7
30 ft						
N	16	15	15	13	11	11
Mean	18.0	21.5	22.7	21.6	22.7	23.8
S.D.	3.1	4.4	3.4	4.4	2.7	3.8
40 ft						
N	20	23	21	16	13	10
Mean	18.9	21.4	23.14	23.8	22.8	24.0
S.D.	3.6	4.4	3.5	4.3	4.0	4.5

N = number of observations.

P = probability that observed rate at 72 min. does not differ from rate at 15 min.

* Difference between 20 and 40 ft significant at 32 min.



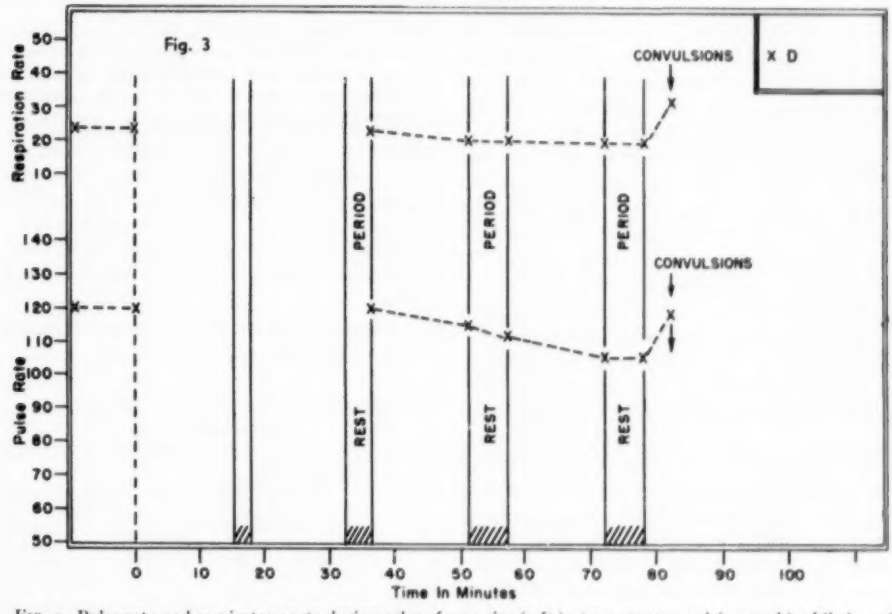


FIG. 2. Pulse rate and respiratory rate during subsurface swim (2 ft.) at constant speed (0.9 mph) while breathing oxygen. (Run was stopped because of symptoms.)

FIG. 3. Pulse rate and respiration rate during runs producing convulsions at a depth of 40 ft.

TABLE 2. OCCURRENCE OF FIXATION OF PULSE RATE DURING RECOVERY PERIOD OR DURING TIME BETWEEN TWO RECOVERY PERIODS

	%
1. Before convulsions (2 cases)	100
2. Before exhaustion end point runs at 40 ft. (2 runs)	100
3. Before symptoms produced in runs at different depths (11 of 14 runs)	78.6
4. Before symptoms produced by long distance surface runs (2 cases)	100
5. Before exhaustion end point by long distance surface runs (2 of 4 cases)	50
6. In normally terminated runs without symptoms or exhaustion (6 of 100 enumerated recovery periods)	6

which symptoms of exhilaration followed by unconsciousness for 10 minutes developed. Figure 3 shows the same phenomenon in the same subject prior to the onset of convulsions at 40 feet. The development of symptoms in this case merits a detailed description.

At the fourth rest period (72nd–78th min.) the subject seemed in good shape. He started swimming and after about three laps (average time for a lap, 24 sec.) he removed his swim fins and mits because of chafing. The irritation the subject felt in hands and feet might have been equivalents of twitching symptoms. He then began swimming more rapidly, crawling with his hands and walking on all fours. He left an unforgettable impression, when he crawled forward plunging his fingers into the drain orifices of the floor plate. It looked as if the whole body was catapulted forward. It was the most unusual picture of a man whose whole will power seemed to have been bundled up and made to an explosive. Could preconvulsive energy be channeled into an apparent coordinated movement? The dramatic motion suddenly stopped and the man had to be lifted from the water and his face mask was removed. A few seconds later, he began a typical grand mal seizure, lasting about 2½–3 minutes, during which he had bilateral clonic movements of the upper and lower limbs. He then began breathing normally and slept 45 minutes. Later he awoke with no apparent ill effects. The pupils were round, regular and reacted at all times.

The second case in which convulsions occurred at 40 feet also showed a fixation of pulse rate prior to the onset of convulsions, and the same phenomenon was noted prior to the exhaustion end point of swims.

Table 2 gives the percentage of cases in which this phenomenon of fixation of pulse rate occurred during transition either from exercise to rest or from rest to exercise.

Table 3 shows that the increased pulse rate in response to swimming between the two last rest periods is significantly smaller in swims terminated because of symptoms than in normally terminated swims.

A comparison of pulse rate and respiratory rate at the end of normal swims with pulse rate and respiratory rate at the end of runs terminated because of the development of symptoms, is given in table 4. Both pulse rate and respiratory rate accompanied by symptoms are significantly higher than those of the normally terminated runs.

Oxygen Consumption. Oxygen consumption data are given in table 5. Values obtained at surface and at 20 feet did not differ at all. A slight increase in oxygen consumption is noted

TABLE 3. INCREASES IN PULSE AND RESPIRATORY RATES PRODUCED BY SWIMMING AT 20, 30 AND 40 FT. FOR 15 MIN. BETWEEN LAST 2 REST PERIODS IN NORMALLY TERMINATED SWIMS (A) AND IN SWIMS TERMINATED BECAUSE OF SYMPTOMS (B)

	A		B	
	Pulse	Resp.	Pulse	Resp.
N	36	22	13	12
Mean	+6.9	+2.95	+1.38*	3.5
S.D.	8.4	3.06	5.05	4.5
P			.05	0.6

* Difference between *Group A* and *B* statistically significant at the 5% level.

TABLE 4. AVERAGE VALUES OF PULSE AND RESPIRATORY RATES

	A End of Normally Terminated Runs (20, 30, 40 ft.; 95 min.)		B End of Runs Producing Symptoms	
	Pulse	Resp.	Pulse	Resp.
N	28	27	13	11
Mean	98.4	23	114.5*	36.3*
S.D.	13.9	2.9	17.78	14.4
P			.004	.001

* Differences between *Groups A* and *B* statistically significant at the .4% level and better.

at greater depths, 30 and 40 feet, which is not statistically significant.

Symptomatology. From a total number of 50 exposures to 20, 30 and 40 feet, 14 were terminated because of the development of symptoms. The percentage frequency of various symptoms, corresponding to the presentation of Yarbrough and associates (1), was as follows: dyspnea, 8; nausea, 3; twitching, 4; vertigo, 0; numbness, 1; irritability, 1; convulsions, 2; visual disturbances, 2.

Dyspnea is by far the most frequent symptom. A closer analysis of the dyspnea symptom showed that most subjects (7 out of 8) had very rapid shallow breathing (rate mostly above 32) and experienced difficulty in "taking a full breath," in "catching the breath" or reported heavy resistance to breathing. Apparently inspiration was inhibited to a certain degree in these cases. One subject, however, who was able to observe his symptoms very conscientiously, perhaps too conscientiously, described an overexpansion of the chest; he had "a feeling in the chest of heavy breathing" when he removed his mask. His respiration rate was only 16 in contrast to the other seven subjects, who showed a much higher respiratory rate.

Relation of Symptoms of Oxygen Toxicity to Partial Pressure of Oxygen and Carbon Dioxide. From 50 exposures to high oxygen, 14 were terminated because of symptoms. The total number of experiments (50) were oriented in order according to the partial pressure of oxygen found at the end of the experiments in the bags of the swimming units and classified into four groups in 10-foot increments. The average concentration measured in the bags at the end of the swims was 79.15% (charged from bottles containing approximately 99% oxygen). Further details are reported elsewhere (5). For each group the percentage of cases with symptoms was obtained. In figure 4 the percentage of symptoms of each group is plotted against partial pressure of *a*) oxygen and *b*) carbon dioxide. The standard deviations are large because of the small numbers in each group. Data seem to indicate that incidence of symptoms of oxygen toxicity is related to partial pressure of oxygen but apparently not to partial pressure of carbon dioxide.

DISCUSSION

Oxygen consumption data were collected in these experiments for the purpose of an estimation of work load. Although the method of oxygen consumption measurement via the pressure drop used in these experiments is considered to give only approximate values, results agree fairly well (table 4) with findings obtained by the cooperative Underwater Project and by Lanphier at the Experimental Diving Unit (17, 18), using an improved pressure-drop method (smaller, 500-cc oxygen supply cylinders, and readings taken more often, once each lap). Our values are lower than those obtained by Donald and Davidson (19) in underwater swimmers.

Our data do not include swims which were terminated because of symptoms. In these cases we were not assured that the pressure reading was taken exactly at the end of the swim, because of the necessity to care for the subject.

Oxygen consumption values indicate that the work load imposed on the swimmers during these experiments was that of moderate exercise (between 1500 and 1700 cc/min.). For an analysis of pulse rate changes, oxygen consumption data are used as a reference index.

TABLE 5. OXYGEN CONSUMPTION DURING UNDERWATER SWIMMING WHILE BREATHING OXYGEN AT VARIOUS DEPTHS*

	At Surface	At 20 ft.	At 30 ft.	At 40 ft.	2–3 ft. Below Surface	At 50 ft. (2.5 atm.)	
Source	MRL	MRL	MRL	MRL	(19)	(17)	(18)
Speed range, mph	.75–.83	91–1.06	.89–1.1	.76–1.1	.89–1.16	.61–.97	1.1
Av., mph	.82	.99	.98	.98			
Time, min.	72–190	75–93	86–120	89–115	20	15–48	
O_2 consumption							
N	8	11	13	11		8	
Mean, cc/min.	1403	1341	1449	1504	2320	1490	1700
S.D., cc/min.	±173	±287	±220	±245	1.16–2.68		

Values from other oxygen consumption studies are listed for convenience of comparison.
* Differences between depths are not statistically significant.

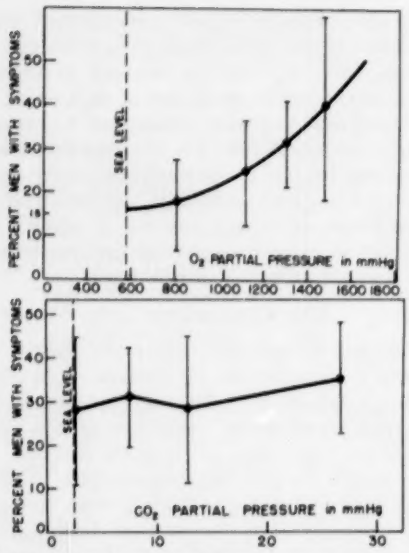


FIG. 4. Relation of symptoms of oxygen toxicity to partial pressure of oxygen and carbon dioxide.

The pulse rate values at the start of the swims were taken after equalization to pressure, while the subjects were standing in the water wearing a respiratory unit and breathing oxygen. Average values at various depths ranged from 93 to 105, and are higher than the average values of 83 (range 60–99) reported by Schneider and Truesdale in their study of 2000 young men (20).

The relation of pulse rates and work load performed on the bicycle ergometer has been found by Schneider and Karpovich (16) to show the following: From an oxygen consumption level of 500 cubic centimeters, which is considered an approximate value for the standing position, pulse rate increased 47% at moderate exercise (1500 cc) and 61% at a somewhat higher work load of 1750 cubic centimeters. For similar work loads in our experiments (1501 cc at 20 feet and 1684 cc at 40 feet) pulse rate increased only 18 and 13% respectively, when using the data obtained at the end of the first 15 minutes of swimming and comparing them with the pulse rate data collected in a standing position prior to swimming.

During underwater swimming while breathing oxygen a strong parasympathicotonic stimulation manifests itself in *a*) highly reduced pulse rate increase compared with similar work loads at a bicycle ergometer; *b*) in a consistent downward trend of the pulse rate during the swims (see fig. 1), and *c*) in end values after 93 minutes of moderately strenuous work which are even below the initial resting values. The cardio-inhibitory action displayed under these conditions is so strong that it leads eventually to a fixation of the pulse rate, or nonresponsiveness of the pulse rate to change from exercise to rest or from rest to exercise. This greatest dominance of parasympathetic activity is found to precede the development of symptoms, which in turn are accompanied by increased sympatheticotonic activity.

The following course of events is found in these experiments: *1st phase*—development of a strong parasympathetic activity; decrease in pulse rate; *2nd phase*—greatest dominance of the parasympathetic activity; fixation of pulse rate, preceding symptoms; *3rd phase*—symptoms, accompanied by increased sympathetic activity.

Phases one and *three* are already known. The demonstration of *phase two* has not been previously reported and promises to be a useful warning sign of acute symptoms. Experienced underwater swimmers can learn to count their pulse rates at the beginning and the end of the rest period. If no change were found they could return to the surface to escape the development of symptoms.

In contrast to exposure to high oxygen pressures under resting conditions, underwater swimming while breathing oxygen produces a much higher frequency of dyspnea among symptoms developed. Rapid, shallow breathing and inspiratory inhibition is the characteristic pattern, which seems to be related to the dominant vagal tone already demonstrated in pulse rate measurements. This finding suggests a possible lead for understanding the amazing reduction in ventilation found with increasing pressure during air or oxygen breathing at a constant given work rate as reported by the NRC Panel on Underwater Swimming (17). It was reported in 1946 by Asmussen and Nielsen that increased oxygen tensions in the inspired gas produce a decrease in pulmonary ventilation during moderately strenuous exer-

cise (21). This effect becomes apparent at oxygen consumption values above 1.2 liters per minute, and has been explained as a predominance of the depressive effect of oxygen on the respiratory center (21). A stimulation of the vagal inhibitory reflex mechanism by high oxygen tensions leading to inspiratory inhibition, as suggested by our experiments, has not been taken into account but might very well play a role in decreasing the pulmonary ventilation during moderately strenuous work. Such a hypothesis is further supported by the finding of Behnke *et al.*, of rapid, shallow breathing alternating with deep respiration in 1 out of 10 subjects exposed to 1 atmosphere of pure oxygen (7).

Attention should be drawn to the fact that under the conditions of underwater swimming the respiratory system more frequently becomes the site of manifestations of oxygen poisoning than under resting conditions.

SUMMARY

Twelve trained underwater swimmers swam at a speed of about .85 knots at surface and at 20, 30 and 40 feet while breathing oxygen for a period of 93 minutes, with rest periods after each 15 minutes.

Average pulse rate, measured at the beginning of rest periods, showed a consistent decline with continuing exercise at each depth, exhibiting the well-known bradycardia effect of oxygen, whereas average respiratory rate showed an upward trend. Average pulse rate and respiratory rate at the end of swims terminated because of symptoms were significantly higher than at the end of normally terminated swims, suggesting that an increased sympatheticotonic activity accompanies symptoms of oxygen toxicity.

Pulse rate measurements at the beginning and end of rest periods made possible the demonstration of a sign of predominant parasympathetic activity, namely the fixation of pulse rate or nonresponsiveness of pulse rate to change from exercise to rest or from rest to exercise. This sign preceded the development of symptoms in 78% of the cases and can be used by the swimmer as a warning for acute symptoms of oxygen toxicity.

Under conditions of underwater swimming while breathing oxygen, dyspnea was usually characterized by rapid, shallow breathing and apparent inspiratory inhibition.

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Spectral Reflectance of the Skin of Rats, Rabbits and Hairless Mice in the Regions 243–700 mμ and 0.707–2.660 μ

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THE DIFFUSE spectral reflectance of living human skin was measured for the visible (1–4), the ultraviolet (3, 4), and the near infrared ranges (5) of the spectrum. Recently, reflectance measurements made in this laboratory, with an improved technique and increased accuracy (6–8), have yielded additional data in the ultraviolet and the near infrared range (3, 9).

Reflectance data are of importance for the study not only of the structure and components of the skin (10, 11) but also of the effects of radiation and other agents which affect the skin (12, 13).

Corresponding reflectance data for the skin of common laboratory animals are rather scarce. Ascolese *et al.* (14) have presented curves on the rabbit, guinea pig and mouse for the range 400–700 mμ; Dimitroff *et al.* (15) have extended the range to 1000 mμ. Helmke (16) has used a limited reflectance measuring method on the living skin of the pig to correlate the visually measured decrease of reflectance in selected spectral bands (around 530 and 660 mμ) with the degree and the time course of radiation-produced erythema and pigmentation.

Reflectance data will furnish the information necessary for judging burn experiments on the basis of absorbed energy rather than of incident energy. The knowledge of the spectral reflectance of animal skin and its comparison with the human skin is important for such investigations.

This report extends the spectral range of earlier measurements on animal skin (15) and presents additional data on the hairless mouse. The spectral range covered in these measurements corresponds to the range of recent measurements on the human skin (3, 9). A corresponding study on the skin of Chester White pigs, used frequently in burn experiments, is under way and will be reported later.

Received for publication August 8, 1955.

METHODS AND MATERIALS

Care and Preparation of Animals. Five Sprague-Dawley albino rats, average weight 225.2 gm; six hairless mice, average weight 19.9 gm; and five New Zealand white rabbits, 15–18 months old, average weight 4260 gm were used in the study. All animals were maintained on Purina Laboratory Chow and water, ad libitum.

The skin area selected for reflectance measurements on the rabbit and rat was the outer aspect of the right thigh; on the hairless mouse it was the posterior dorsal portion of the animal. The selected areas were prepared for spectral reflectance measurements as follows: rabbit and rat, clipped and depilated with Imra depilatory cream; the mouse, void of hair, needed no preparation. The measurements on the rats were made immediately after preparation, whereas on the rabbits they were made 48 hours after depilation because the depilating agent gave rise to some erythema after 24 hours (15).

All animals were anesthetized with Sodium Nembutal (Abbott), dosage as follows: rabbit, 25 mg/kg, intravenously; rat, 50 mg/kg, intraperitoneally; hairless mouse, 62.5 mg/kg, intraperitoneally.

Reflectance Measurements. *1. 243–700 mμ range.* As in the experiments on human skin (3), the Beckman Model DR recording spectrophotometer, equipped with a comparison type integrating sphere (8), was the instrument used for this spectral range. A $MgCO_3$ block which had previously been standardized against a 2-mm layer of MgO on a front surface mirror was used as the working standard (8). The measured reflectance values were converted to reflectances relative to the reflectance of MgO (i.e. r_{skin}/r_{MgO}) and are so reported.

2. 0.707–2.660 μ range. The instrument used for this spectral range was the NML spectrophotometer assembled at the Naval Material Laboratory (5) and modified with a comparison type integrating sphere designed and built at this laboratory (7). The same instrument was used previously for the measurements on human skin over the same wavelength range (9).

A vitrolite sample which had been standardized against MgO was used as a working standard. Two recordings were made, one with the beam incident on the vitrolite and the other with the beam incident on the skin. The measured reflectance values were converted to reflectances relative to the reflectance of MgO (i.e. r_{skin}/r_{MgO}) and are so reported.

RESULTS

1. 243–700 mμ Range. In figure 1, *curve 1* shows the mean spectral reflectance of six hairless mice, *curve 2* of five New Zealand white

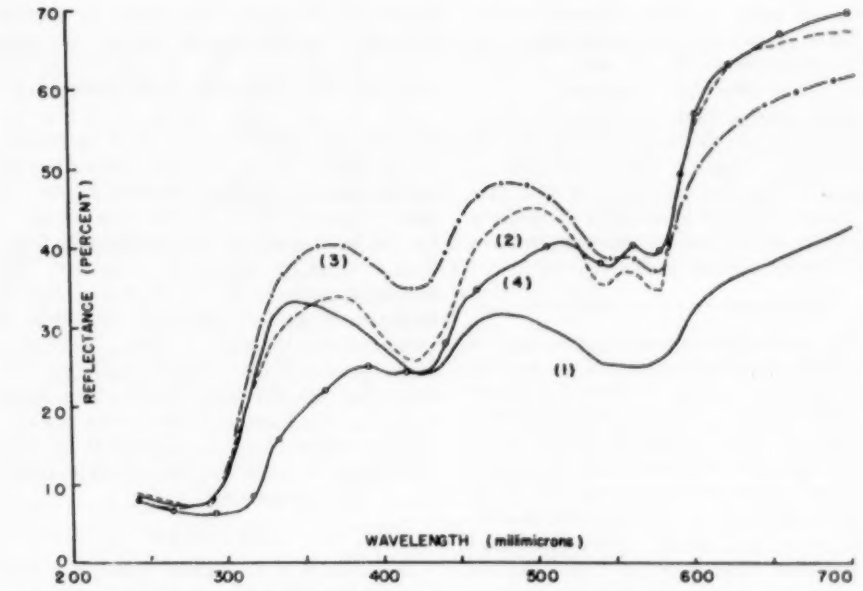

FIG. 1. Spectral reflectance values of the skin of hairless mice (*1*), rabbits (*2*) and rats (*3*) (mean values); and of human skin (*4*) (a fair-complexioned individual).

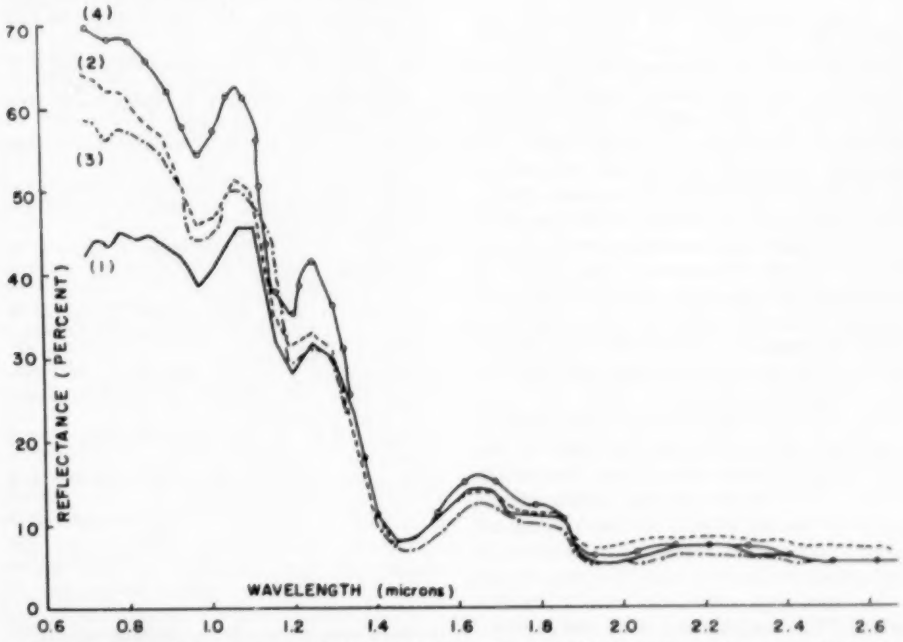

FIG. 2. Spectral reflectance values of the skin of hairless mice (*1*), rabbits (*2*) and rats (*3*) (mean values); and of human skin (*4*) (a fair-complexioned individual).

rabbits and *curve 3* of five Sprague-Dawley rats. *Curve 4*, showing the spectral reflectance of fair-complexioned human skin, obtained earlier (3) was added for comparison.

2. 0.707–2.660 μ Range. In figure 2, *curve 1*, the mean spectral reflectance of the six hairless mice is given. *Curves 2* and *3* show the reflectance of the five rabbits and five rats, respectively, and *curve 4*, added for comparison, shows the reflectance of fair-complexioned human skin as reported earlier (9).

DISCUSSION AND CONCLUSIONS

In the wavelength region 243–700 mμ, the shapes of the reflectance curves of the animals studied are quite similar to that of a lightly pigmented human, as can be seen in figure 1. Below 300 mμ there appears to be little or no difference in spectral reflectance measurements on either the animals studied or on humans as previously reported (3). As a percentage comparison, values obtained on the rat, rabbit and hairless mouse range from 7.2–8.7%, whereas values obtained on the human skin were from 6 to 8% and show little variation among individuals. In the wavelength range 300–425 mμ, the values obtained for the hairless mice and rabbits do not differ appreciably, whereas values obtained on the rat are slightly higher. Above 425 mμ the values obtained for the hairless mouse are considerably lower than the reflectance values of the rat or rabbit. As previously reported (15), the mean skin thickness of the rabbit and rat is 1.8 and 1.0 mm, respectively. Measurements of the thickness of the hairless mouse are of the order of 0.6 mm. Because of this difference in skin thickness of the mice in comparison with the rats and rabbits, it can be assumed that reflectance values obtained on the hairless mice do not represent the reflectance of the skin only but also of deeper tissues.

The prominent absorption bands of hemoglobin and water are quite evident for the rabbit and rat and comparable to those of the human. The oxyhemoglobin bands appear clearly in the rabbit and rat but are not resolved in the hairless mouse. This again can be ascribed to the contribution of deeper tissues to the measured reflectance of the hairless mouse. This would mean that the spectrophotometric reflectance measurement is not well suited to study skin effects on the hairless mouse. On the other hand, it may be possible that on this thin-skinned animal the non-distinctive method may be helpful in the observation of changes in deeper tissue areas.

For the wavelength region, 0.7–1.2 μ, the shapes of the reflectance curves of the hairless mouse, rabbit and rat are quite similar to that of the fair-complexioned human as can be seen in figure 2. However, the values obtained for the hairless mouse are considerably lower than those of the rat, rabbit or human. This difference may again be attributed to the difference in skin thickness of the mouse as compared with the rat and rabbit. Above 1.2 μ there appears to be very little difference in skin reflectance values obtained on the animals studied or the human, as previously reported (9). The values of 5–8% obtained at the long wavelength limit may be due to Fresnel reflection at the skin surface.

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Relationship Between Serum Amino Acid Concentration and Fluctuations in Appetite[1]

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A NUMBER of observations suggest that amino acid metabolism may have something to do with the regulation of hunger. If amino acid solutions are infused too rapidly, anorexia or nausea may appear (1, 2), and gastric peristalsis has been found to cease during the intravenous administration of amino acids (3). In this laboratory measurements of the serum amino acid and blood sugar concentrations have been made under a variety of circumstances (4, 5). Simultaneously, crude estimations of appetite have been attempted.

METHOD

Just before each blood sample was taken the subject was asked whether or not he was 'hungry.' An attempt was made to interpret his response according to the following scale: nauseated, minus 1; no desire for food, 0; might eat if offered food, but not very hungry, 1 plus; ravenous, 4 plus; and 2 plus and 3 plus graded between 1 plus and 4 plus.

In all of the experiments reported here the subjects were either normal volunteers or patients with no disease known to affect protein or carbohydrate metabolism. In *experiment I* nine subjects ate a standard breakfast containing approximately 20 gm of protein: two eggs, a glass of milk and a piece of toast. Venous blood was collected in the fasting state and at hourly intervals after the breakfast for 4 hours. The serum amino acid nitrogen concentration of each blood sample was determined in duplicate by the method of Albanese and Irby as previously described (4). The standard deviation in 230 such pairs was 0.16 mg %.

In *experiment II*, with 11 subjects, 500 cc of 5% amino acids and 5% glucose[2] were infused in 45 minutes. Blood specimens were obtained in the fasting state and at hourly intervals for a period of 4 hours after the beginning of the infusion. In five instances blood samples were also obtained ½ hour after the start of the infusion. Serum amino acid nitrogen was determined as described above, and the blood sugar concentration of each sample was determined by the Mattice modification of the Folin-Wu method (6). For 286 such paired determinations the standard deviation was 2.7 mg %.

In *experiment III*, with 13 subjects, 250 cc of a 10% aqueous solution of enzymatically digested casein[2] were infused in 45 minutes. Blood specimens were obtained immediately preceding the infusion and after the beginning of the infusion at the following intervals: 10 minutes, 30 minutes, 1 hour, 2 hours, 3 hours and 4 hours. Blood sugar concentration of each specimen was determined as described above, and serum amino acid concentration of each specimen, except those drawn at 10 and 30 minutes, was estimated as described above.

In *experiment IV* each of 13 subjects drank 250 cc of the same 10% amino acid mixture used in *experiment III*. Both serum amino acid nitrogen and blood sugar were estimated in the fasting state and at ½-hour intervals for 2 hours and for another 2 hours at 1-hour intervals.

RESULTS

For a long time we were unable to decide how these data could best be analyzed. In fact, it seemed unlikely that such crude estimations of the desire to eat could be tabulated or pooled at all. Nevertheless, simple graphic representation of the data from individual subjects seemed usually to follow this pattern: in all four experiments the appetite diminished when the amino acid concentration rose, and when the amino acid concentration fell the appetite increased. This tendency is illustrated in figures 1 and 2. Unfortunately, blood sugar determinations were not made in *experiment I* (test meal), but in the three experiments in which the blood sugar concentration was measured there seemed again to be a general pattern (figs. 1 and 2): when amino acids were infused with glucose, the appetite appeared to vary inversely with both the amino acid and blood sugar concentrations, but when amino acids were administered alone, whether by mouth or by vein, the appetite appeared to

Received for publication August 29, 1955.

[1] This investigation was supported by a research grant from the United States Office of Naval Research, Contract Nonr-233(26).

[2] Five per cent Amigen and 5% glucose and 10% Amigen generously supplied by Mead Johnson and Co.

[2] Ten per cent Amigen generously supplied by Mead Johnson and Co.

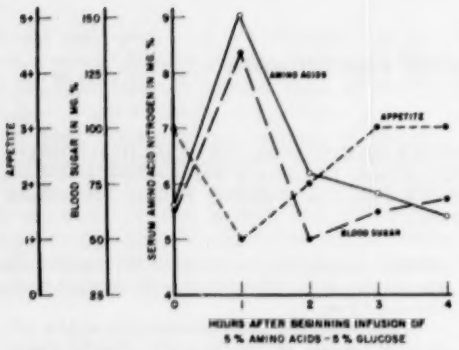

FIG. 1. Appetite and blood sugar and serum amino acid concentrations in *JK* following infusion of 500 cc of distilled water containing 5% amino acids and 5% glucose in 45 min.

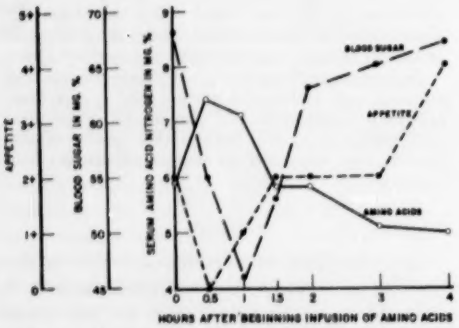

FIG. 2. Appetite and blood sugar and serum amino acid concentrations in *AR* following infusion of 250 cc of 10% amino acids in 45 min.

vary inversely with the amino acid concentration but directly with the blood sugar.

There was considerable variation, however, from subject to subject, and not all of the appetite responses behaved in the manner described above.[4] Each individual's fasting 'appetite level' was arbitrarily called 0, and deviations from this point were measured in terms of the number of 'plusses' separating any subsequent 'appetite level' from the fasting one. Thus, if *subject 1* had been thought to be 2 plus hungry in the beginning, and 0 at 1 hour, the appetite level at 1 hour was called minus 2. In the same way each fasting amino acid and sugar concentration was called 0, and deviations from this point expressed in mg % plus or minus. Correlation coefficients were then calculated for the association of fluctuations in appetite with changes in the amino acid and sugar concentrations (table 1). There were statistically significant negative correlations between changes in the serum amino acid nitrogen concentration and appetite in all four experiments. In *experiment II* (i.v. amino acids with glucose) there was a negative correlation between changes in the blood sugar concentration and the appetite, but in *experiments III* and *IV* (i.v. and oral amino acids) there was a positive correlation between changes in the blood sugar concentration and appetite. Some of these data are depicted in a scatter graph (fig. 3).[5]

DISCUSSION

Whether induced by feeding of protein or of amino acids, or by infusing amino acid mixtures, a rise in the serum amino acid concentration appears to be accompanied by a waning of appetite. The subsequent increase of appetite is accompanied by a fall in the amino acid concentration. Although the measurements of appetite were very rough and undoubtedly inaccurate, it seems unlikely that the correlations found here so consistently would have occurred by chance. Inaccuracy in measuring appetite would not of itself produce such correlations, unless the errors were somehow similar and consistent. The latter possibility seems very remote indeed, since almost all of the subjects knew nothing about the objectives of the study, nor were they in any way prejudiced as to the type of response expected.

When in *experiment I* it appeared that appetite varied in a direction opposite to the serum amino acid concentration, it seemed possible that factors responsible for appetite and the normal emptying of the stomach were correlated, and that the emptying of the stomach in turn regulated the rate at which protein was digested and absorbed as amino acids. If

[4] We are indebted to Dr. Victor Hall for suggesting a method that proved helpful in the statistical analysis of all the data.

[5] Five additional figures have been deposited as document number 4767 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D. C. Copies may be secured by citing the document number and by remitting with the order $1.25 for photoprints, or $1.25 for 35 mm microfilm. Make checks or money orders payable to: Chief, Photoduplication Service, Library of Congress.

this hypothesis were correct, the amino acid blood level per se would have nothing to do with appetite. In *experiment II*, however, it became evident that intravenously induced amino acid blood levels were similarly related to appetite, and hence the emptying time of the stomach would not seem to be an essential factor in these relationships.

In *experiment II* (amino acid-glucose infusions) the appetite appeared to diminish with a high blood sugar concentration and to flourish with low blood glucose levels. Such an association was in keeping with classical ideas about the blood sugar and hunger (7). The correlation coefficient relating blood sugar and appetite was less (−0.318) than the correlation coefficient relating the serum amino acids to appetite (−0.405). Nevertheless, the possibility remained that the causal correlation was between the blood sugar and the appetite, and that the amino acids happened to wax and wane along with the sugar. To test this hypothesis a method was needed to make the blood sugar and the amino acids change in opposite directions.

This opportunity presented itself with the chance discovery that the infusion of amino acids created a reciprocal relationship between the serum amino acid and blood sugar concentrations (5). Accordingly, it was found in *experiments III* and *IV* that the administration of hydrolyzed casein by vein or by mouth diminished appetite as the amino acid concentration rose and the blood sugar fell. That the appetite should be at its nadir when the blood sugar concentration was low is particularly surprising because some of the blood sugars attained were below 40 mg % and produced signs of hypoglycemia, such as sweating, torpor and tachycardia. Similarly, the recovery of appetite was associated with a fall in the serum amino acids and a rise in the blood sugar.

TABLE. 1. CORRELATION BETWEEN FLUCTUATIONS IN APPETITE AND CHANGES IN SERUM AMINO ACID AND BLOOD SUGAR CONCENTRATIONS

Exp.	r* for Change in Serum Amino Acid Nitrogen Conc. and in Appetite	*P*	r* for Change in Blood Sugar Conc. and in Appetite	*P*
1	−0.595	<0.01		
2	−0.405	<0.01	−0.318	<0.05
3	−0.500	<0.01	+0.232	<0.05
4	−0.526	<0.01	+0.297	<0.05

* Correlation coefficient.

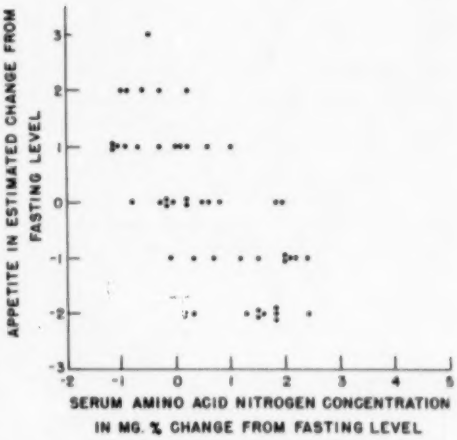

FIG. 3. Relationship between change in appetite and change in serum amino acid concentration in 13 subjects following ingestion of 250 cc of 10% amino acids.

These data by no means prove that the blood sugar has nothing to do with the control of appetite. Neither do they establish a causal relationship between the serum amino acid concentration and the appetite. As a matter of fact, it seems unlikely that the serum amino acid concentration per se is an important determinant of appetite because the fasting amino acid concentration does not reflect the degree of hunger. Diabetics, for example, have in general a relatively high fasting blood amino acid concentration (8), despite the well-known diabetic polyphagia. In hepatitis, a disease notorious for anorexia, the postprandial serum amino acid concentration is relatively low (4).

How, then, can these relationships be explained? Only further experiment can answer this question. At present one can only observe that after the consumption of protein some metabolic change associated with waxing and waning of the serum amino acid concentration appears to affect appetite, and that this effect seems to be independent of changes in the blood sugar concentration.

SUMMARY

In normal volunteers and patients without diseases known to affect protein or carbohydrate metabolism crude estimations of

appetite were correlated with the serum amino acid and blood sugar concentrations in four circumstances: *a*) after a 20-gm protein breakfast, *b*) after infusions of hydrolyzed casein and glucose, *c*) after infusions of hydrolyzed casein alone and *d*) after the ingestion of hydrolyzed casein. In all four experiments there was a reciprocal relationship between the serum amino acid concentration and appetite. A similar relationship between the blood sugar concentration and appetite was found after infusions of glucose and hydrolyzed casein, but the administration of hydrolyzed casein alone caused the blood sugar concentration and the appetite to diminish simultaneously.

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Effect of Water Content and Compression on Clothing Insulation

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MODERN military aircraft missions over arctic seas require clothing suitable for the protection of aircraft crew members against the hazards of emergency exposure to extremely cold water. The serious and often fatal hypothermia resulting from such exposures was indicated by the work of Spealman (1), Newburgh and Spealman (2, 3), Molnar (4) and Wayburn (5).

Emergency exposures may occur under conditions where individuals wearing outer water impermeable suits have accumulated considerable amounts of sweat within their clothing. Furthermore, accidental leakage of the cold water during the immersion period may occur. In both cases a reduction of thermal insulation results, with a consequent decrease in tolerance time. Although some data on relative heat loss of man in air and water exist (4, 6), effects of water compression and clothing water content on thermal insulation have not been quantitatively defined. Griffin *et al.* (7) reported a decrease in thermal insulation when sweat or small amounts of water were added to clothing, but these studies did not include water immersion.

The reported study was undertaken to quantitatively determine: *a*) the effect of water compression on clothing insulation; *b*) the relation between clothing water content and thermal insulation in both air and water; *c*) the effect of wetting small body areas (feet) as compared with large areas (trunk, arms, legs), and *d*) the relative heat loss of a clothed manikin in air and water when wearing dry or wetted clothing. These experimental results permit the calculation of a predicted tolerance time in water at 0°C (and other temperatures also) as a function of the clothing insulation or water content at various metabolic levels.

PROCEDURE

Thermal insulation of complete clothing assemblies was measured with a sitting model, copper manikin and insulation of the footwear (sock) by means of a copper thermal foot. A total of 67 experiments was conducted, including 12 in connection with the wet sock study. Thermal insulation is expressed in terms of clo[1] units.

A description of the various clothing components worn is included in table 1. In figure 1 the position of the fully dressed manikin in water is shown, and is typical of the immersion depth used in all the water measurements of insulation. Water temperature (13–18°C) was continuously measured with copper-constantan thermocouples and recorded on a Leeds and Northrop Micromax. Variation in water temperature did not exceed ±0.5°C in any one test, and the water was constantly stirred with a stream of compressed air.

Insulation of Ambient Air (I_a). Since insulation of the air layer surrounding the manikin is a component of the total or effective insulation, these measurements were required if total thermal insulation of clothing in air was to be considered. These experiments were conducted with the nude manikin. Results obtained under two different ambient air temperature (and consequent air movement) conditions were:

Ambient Temp.	No. Tests	I_a-clo (av.)
14°C	3	0.47
27°C	3	0.65

These values, used in the air measurements of thermal insulation, are reflected in the values presented in the summary of all experiments (table 2). Values for I_a in the case of the copper thermal foot where experiments were performed in a different test room varied from these values slightly, the average (I_a) value for the nude foot being 0.45 equivalent clo.

Air Measurements—Dry and Wet Clothing. Measurements of clothing insulation in air, and with the manikin dressed in dry clothing, were first conducted by methods previously described (9) and routinely used for copper manikin models. Measured quantities of water were then added to the clothed trunk, arm and leg areas of the manikin, and the water-impermeable (R-1) outer exposure suit immediately placed over the wetted clothing. A specially improvised neck closure prevented loss of any significant quantity of water during the insulation test period. All clothing items were weighed before and after measurements of thermal insulation, and water content figures are based upon

Received for publication July 20, 1955.

[1] The clo is a unit of insulation defined as the insulation necessary to maintain in comfort a sitting-resting subject in a normally ventilated room when air movement is 20 ft/min., ambient air temperature is 21°C and humidity is less than 50%.

TABLE 1. CLOTHING INSULATION WORN

Clothing Assembly Tests

1. Two-piece cotton-wool (50-50) underwear, medium-regular
2. USAF anti-G suit, G-4B, medium-regular
3. USAF ventilating garment, MA-1, regular
4. Insulation liner (Navy Type), for Mk-IV anti-exposure suit, size 40
5. Wool knit glove inserts
6. Medium weight wool socks
7. Bristolite (10D) sealed insulation boots
8. USAF anti-exposure suit, Type R-1

Wet Sock Tests

9. Cushion sole sock, size 10
10. Vinylite sock, outer and inner
11. QM leather shoe, size 9D

amounts present at the conclusion of each test. In these air measurements, the total, or effective, thermal insulation is equivalent to the sum of garment (I_g) and air (I_a) insulations, thus $I_{effective} = I_g + I_a$.

In these air tests the outer clothing worn was impermeable since this was required in the water tests to prevent leakage. The relative impermeability of the outer clothing to air practically eliminated air permeability as a factor in the measurement of the clothing insulation in air. Air movement was also relatively low, being less than 200 ft/min. in all tests of this type.

Water Measurements—Dry and Wet Clothing. Thermal insulation of the dry clothing was measured by immersing to the neck level the fully dressed manikin in a tank of constantly stirred water. In this position approximately 92% of the total manikin area was immersed (i.e. 1.74 m²). Manikin position for these clothing insulation measurements in water is shown in figure 1. Method for adding water, test procedure and method in general for the water measurements were similar to those used for the air measurements. However, in water the total, or effective, insulation is represented only by garment insulation (I_g); thus I (effective insulation) equals I_g.

Wet Sock Measurements in Air. The effect of wetting a relatively small body area (sock or foot, 0.07 m²) was studied in a series of tests conducted on a thermal copper foot. In these tests a cushion-sole sock was placed between two very thin water-impermeable rubber socks and this entire combination (rubber cushion-rubber sock) then fitted within a standard type Army issue leather shoe (12EE) which in turn was fitted upon the copper thermal foot. Dry insulation of this footgear assembly was then determined by the described method. Measured quantities of water were then added to the sock and thermal insulation at the various degrees of wetting determined. Degree of sock wetting varied from 10 to 90% of its saturation value.

RESULTS

A. Water Content and Insulation of Clothing Assemblies—Air and Water. A summary of the results of the insulation measurements of dry and wet clothing obtained in air and water is presented in table 2. Measurements of the effect of clothing water content on the insulation of complete clothing assemblies (large wetted area) were conducted with two objectives in view: *a*) to simulate effects of sweating as represented by the determination of wet clothing insulation in air and *b*) to simulate water leakage and/or sweating effects, these conditions being represented by the determination of wet clothing in water. In figure 2 the effects of water content on thermal insulation (clo) of clothing are plotted. Curves for both effective insulation in air ($I_g + I_a$) and in water (I_g) are given, and effects obtained with large wetted body areas compared to those observed when small areas (socks) are similarly wetted. In figure 3 average percentage loss of thermal insulation (I_g) with water content is plotted for complete clothing assemblies in air and water and for the footwear in air. The similarity of the curves obtained for large wetted clothing areas (assemblies) in either air or water, and with the small wet clothing areas (socks) is indicated.

B. Water Content and Thermal Insulation of Socks—Air. The relationship between water content and thermal insulation of small wetted body areas (socks) is illustrated in figures 2

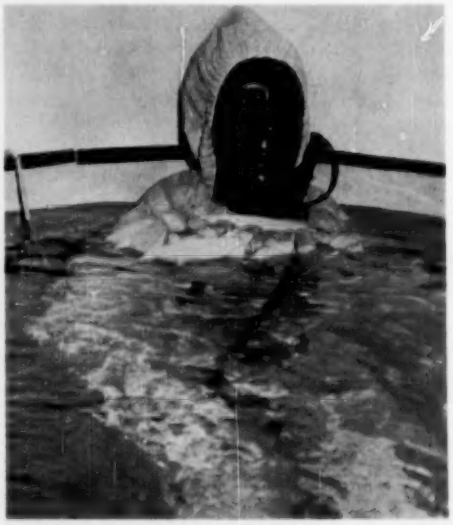

FIG. 1. Copper manikin position for clothing insulation measurements in water.

and 3. In these graphs the general similarity of wetting small as compared with large areas may be noted. Precision of measurement and technique for wetting the small sock areas was generally more satisfactory than was the case for the larger areas. In the range 0–200 gm/m² the effects of wetting large and small areas are quite similar, and even in the higher water content range (200–1000 gm/m²) differences between the two areas did not exceed 14% at any equivalent wetness. Detailed results are included in table 2.

C. Water Compression and Thermal Insulation. Although loss of thermal insulation in water immersion due to hydrostatic pressure is obviously to be expected, quantitative information concerning this effect has previously been lacking. In figure 2 comparison of the effective clothing insulation value when measured dry, in air (3.36 clo), with that when measured dry in water (1.45 clo) shows the significant loss of insulation due to water compression. Effective or total insulation was reduced from 3.36 to 2.26 clo by maximal wetting (940 gm/m²) of the clothing; in water, however, the same clothing assembly, wet to approximately the same extent (1051 gm/m²), total or effective insulation was reduced to 0.73 clo, a total loss in potential insulation of 78.3%. Thermal insulation loss due to compression of the dry clothing by immersion in water was more significant, it may be noted, than loss due to water content over the range of wetness measured.

TABLE 2. INSULATION MEASUREMENTS OF DRY AND WET CLOTHING IN AIR AND WATER

No. Tests	Water Content gm/m²	Insulation-clo	
		$(I_g + I_a)$	(I_g)
	Assembly, Air		
3	0	3.36	2.71
	Assembly (Exp. Liner), Air		
3	0	2.83	2.18
	Assembly, Air		
2	181	3.27	2.62
2	207	2.79	2.14
2	229	2.07	2.42
5	312	2.81	2.24
2	377	2.53	1.87
3	403	2.50	1.97
1	476	2.77	2.12
2	518	2.58	2.11
3	652	2.46	1.99
5	764	2.49	2.02
2	940	2.26	1.79
	Assembly, Water		
3	0		1.45
	Assembly (Exp. Liner), Water		
2	0		1.11
	Assembly, Water		
2	99		1.36
2	222		1.24
2	337		1.18
2	362		1.05
2	436		1.03
2	690		0.94
2	1051		0.73
	Sock, Air		
4	0	1.06	0.62
	Sock, Air		
2	127	0.99	0.53
2	549	0.91	0.44
2	1099	0.86	0.37
2	1268	0.79	0.32

D. Relative Heat Loss of Clothed Manikin in Water and Air. The preceding measurements of resistance to heat flow, or insulation, on the copper manikin in air and water, with both dry and wet clothing, also permitted the measurement of the relative heat loss ratio water/air. In figure 4 the relative heat loss of the clothed manikin in water and air is plotted for dry and various wetted clothing conditions. This ratio varied from 2.3 for dry clothing to 4.0 for the maximally wet clothing. The curves plotted were based upon the assumption of an equivalent initial mean skin temperature (33.3°C). As the heat loss ratio values indicate, cooling in water compared to air increases significantly as clothing becomes wet. The calculated values of 2.3, 2.9 and 4.0 were checked by copper manikin measurements of heat loss in air vs. water at equivalent temperature gradients. Mean values of these measurements (2 or more tests) are also shown in figure 4.

E. Predictions of Human Tolerance Time at 0°C Water Temperature. With the relationship between insulation and clothing water content of a protective clothing assembly in both water and air defined, a further extension of the data permits the prediction of human tolerance time in a condition of extremely cold water (0°C). These predicted tolerance curves were based upon the following assumptions: *a*) Body surface area = 1.8 m²;

b) mean terminal skin temperature of 24°C for water exposures; 30°C for air exposures; *c*) net body heat storage at start of immersion = 0 Cal/m²/hr.; *d*) air or water temperature = 0°C; *e*) body heat storage limit of 50 Cal/m²/hr.; i.e. 90 Cal/hr. for the man; *f*) average metabolic heat increase, due to cold water exposure, 50%; this was included in the calculated metabolic rates. Assumptions *b* and *f* with respect to cold water immersion are based on previously published data (9) and with respect to air exposures assumption, *b* is based upon the results of Taylor (10) which indicated the above-selected heat debt as sufficient to bring an individual to a point of uncomfortable cold and shivering. While the limitations of some of the assumptions are clearly realized, these tolerance time predictions furnish reasonably realistic information of a type previously lacking.

In figure 5 tolerance times in water at 0°C at metabolic levels of 75, 85 and 112.5 Cal/m²/hr. are plotted as functions of clothing water content. The influence of higher metabolic level on tolerance time is indicated.

In figure 6 a comparison of net body heat storage changes in air and water at 0°C with both dry and wet clothing at various metabolic levels is graphically presented. The assumed heat loss tolerance limit (90 Cal/hr.) is indi-

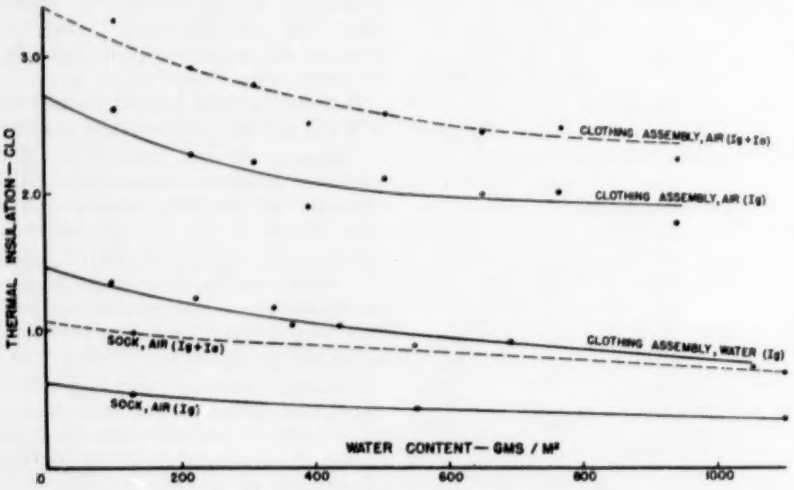

FIG. 2. Effect of water content on thermal insulation.

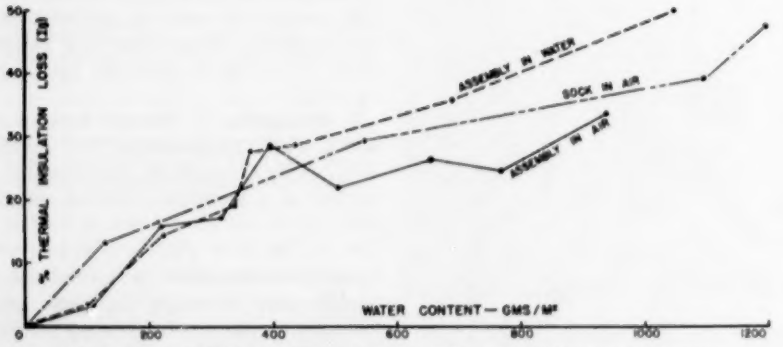

FIG. 3. Percentage loss in thermal insulation (I_g) of wet clothing in air and water.

cated by a dashed horizontal line on the graph. Data of this graph, it should be mentioned, apply specifically to the type protective clothing or assembly used in these measurements or to clothing assemblies having closely similar insulation values (3.4 clo). In water, for example, with dry clothing and a metabolic level of 75 Cal/m²/hr. a tolerance time of 3 hours is indicated. With a lower metabolic level (50 Cal/m²/hr.) tolerance time is reduced to 72 minutes. The marked difference between air and water tolerance times at equivalent metabolic levels, particularly if clothing is wet, is illustrated.

DISCUSSION

Previous studies of Griffin *et al.* (7), Spealman (1) and Newburgh and Spealman (2, 3) demonstrated the serious effects of moisture, either in the form of accumulated sweat, or as water leakage on clothing insulation. However, in these previous investigations the effect of clothing compression by hydrostatic pressure in conjunction with clothing wetness was not considered. A man immersed in water to the neck level has approximately 92% of his clothing under some compression load, and a significant (56.8%) loss of thermal insulation was observed.

Experience of many investigators under arctic conditions conclusively pointed out the hazard to survival which moisture or sweat accumulation within clothing poses. Except for the work of Griffin *et al.* (7), however, few quantitative data exist concerning the relationship between clothing thermal insulation (clo) and clothing moisture content. In cold water immersion, maintenance of dry insulation by preventing water leakage and by minimizing accumulation of sweat within the clothing is especially important for survival. In these experiments both large (complete clothing assembly) and small wetted areas (socks) were studied, and in figure 3 the general similarity of the curves of insulation vs. water content for the respective areas is demonstrated. The effect of water content on clothing insulation, whether measured in air, or in water where compression loads are present, was also similar. Variation in the pattern by which the water was added to the clothing did not significantly alter the results. Quantity rather than distribution of the water appears the more significant factor in respect to effect on clothing thermal insulation. Although small quantities (100 gm/m² or less) do not critically reduce clothing thermal insulation, the effect of greater water content was progressive, and at the maximal degree of wetness (900 gm/m²) effective insulation was reduced to approximately one-half the dry value.

Estimations of heat losses in water reported by Aschoff (6) were based on calorimetric measurements of heat loss of the hands only. More recently Molnar (4) compared entire body cooling in air and water based on data taken from the nude man in air and water at 4.4°C (40°F) after 1 hour of exposure.

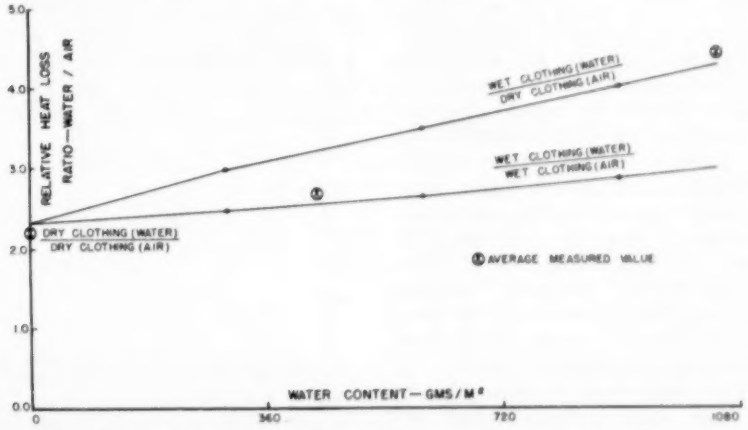

FIG. 4. Relative heat loss of clothed copper manikin in water and air.

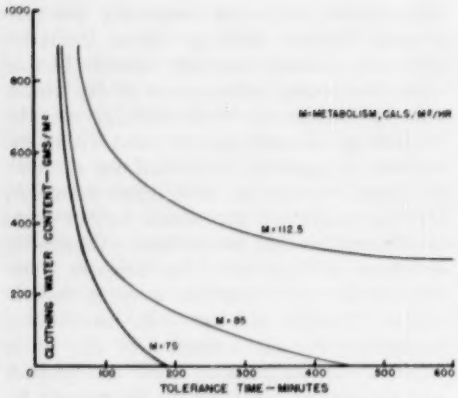

FIG. 5. Predicted tolerance time in water at 0°C as function of clothing water content.

These estimates indicated that heat loss from the body is about twice that in air, or only one-tenth that expected on the basis of water/air conductivities. As pointed out, there were some errors involved in these estimates but not great enough to bring heat loss in water to more than three or four times that in air. Greater effective body surface area for heat loss in water, together with the greater specific heat of water, was cited as the chief reason explaining these estimated heat loss increases in water.

Measured relative heat losses in these experiments confirm the estimated values of Molnar. The manikin dressed in dry clothing lost heat 2.3 times more rapidly in water than in air; with clothing maximally wetted (900 gm/m²) the rate of heat loss in water increased to 4.0 times the heat loss rate in air. The measured heat losses of the clothed manikin in water and in air were performed under equilibrium conditions, i.e. with a constant temperature gradient between copper manikin skin surface (T_s) and ambient water, or air (T_a). In human exposure to cold water, however, vasomotor response and metabolic stimulation due to shivering are of course complicating factors. However, assuming temperature equilibrium conditions, the above measured differences in cooling rates in water and air should be closely approximated.

With the relationship between clothing insulation and water content defined, predictive curves of human tolerance based on an assumed body heat debt (50 Cal/m²/hr.) in water at 0°C were plotted. In these calculated tolerance curves two characteristic events of cold water immersion, namely, the rapid cooling of the skin even in clothed individuals and the stimulation of metabolism due to shivering were considered. Previous results in cold water immersion experiments with human subjects wearing comparable insulation (9) indicated a mean terminal skin temperature of 24°C and a 50% increase in metabolic level for exposures of reasonable duration (1–2 hr.). These characteristic responses of clothed humans to cold water immersion are thus included in these predictions of tolerance time. The sharp drop in skin temperature was always observed; however, wide individual variation as regards onset and severity of shivering occurred, and the metabolic increase used above is conservatively based on a minimal shivering response. As illustrated (figs. 5 and 6) metabolic level and dry insula-

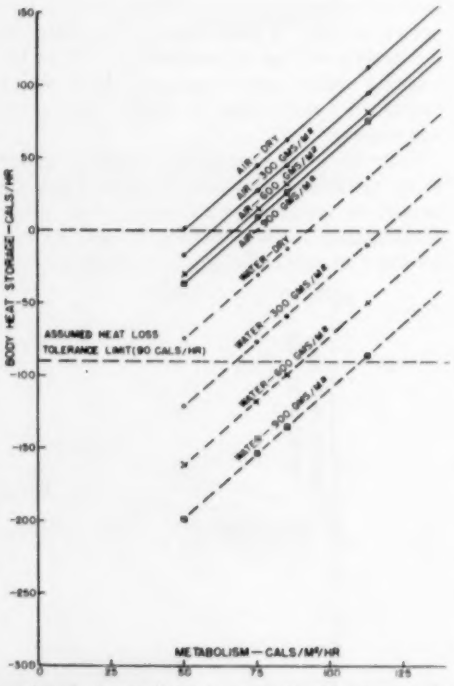

FIG. 6. Net body heat storage in air and water at 0°C with dry and wet clothing at various metabolic levels.

tion are both critical factors in determining tolerance time in cold water. The importance of preventing water leakage and maintaining high metabolic level for maximal tolerance time in extremely cold water is therefore obvious. At any given metabolic level the advantage of maintaining dry insulation and the relative severity of water vs. air exposure is clearly demonstrated (fig. 6).

The assumption of a body heat debt limit of 50 Cal/m²/hr. is based on heat losses observed in cold air exposures (10). Undoubtedly some individuals will tolerate larger heat debts safely. However, for purposes of establishing practical and realistic tolerance times for all types of individuals, minimal shivering responses and some clothing water content may be assumed. In the use of these predicted tolerance curves, the preimmersion metabolic level, clothing water content and immersion water temperature must be known or reasonably estimated before reliable tolerance or rescue times are to result. Assuming a metabolic level of 75 Cal/m²/hr. and dry clothing, these results confirm a previous estimation (8) of 2–3 hours' tolerance time in water at 0°C.

SUMMARY

The effect of water compression (resulting from immersion to the neck level), simulated water leakage and sweat accumulation upon thermal insulation of a typical Air Force protective clothing assembly was determined in a series of 55 experiments conducted with a copper manikin. Results of a series of tests on the effect of simulated sweat on thermal insulation of footwear (sock) are also included. Insulation loss due to hydrostatic compression and removal of the ambient air insulation (I_a) by water immersion was significant, amounting to 56.8%; addition of water in quantities ranging from 100 to 1000 gm/m² caused further reduction of thermal insulation, reaching a maximum of 21.8% at 1000 gm/m². Percentage loss of thermal insulation with increasing water content, whether simulating leakage (measurements in water) or sweat accumulation (measurements in air), was similar. Comparable effects of water upon the thermal insulation of footwear were also observed in a series of 12 tests conducted on a copper thermal foot. On the basis of a total body heat storage loss of 50 Cal/m²/hr., predictive curves for human tolerance time in water at 0°C as functions of clothing water content, metabolic level and thermal insulation are presented. Measured heat loss of the clothed manikin in water was 2.3 to 4.0 times greater than in air, depending upon whether clothing was dry or maximally wetted (1000 gm/m²).

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Body Temperature of the Fairy Prion (Pachyptila Turtur) in Flight and at Rest[1]

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ALTHOUGH the published investigations of avian body temperatures are now rather extensive, very little attention has been given to the effect of flight on thermoregulation. Groebbels (1–4) has reported that migratory birds of several passerine species taken from nocturnal flight at the lighthouse on Helgoland had cloacal temperatures which were usually in the order of 1–2° lower than those of the same species taken in traps during the day. The latter presumably had not been involved in prolonged flight immediately prior to capture. Groebbels (2) was of the opinion that the lower body temperatures of these nocturnal migrants could not be explained alone on the basis of the normal diurnal cycle but that they were actually a reflection of an altered metabolic state characteristic of migration. Merkel (5) has reported that body temperatures in several species during periods of *Zugunruhe* (nocturnal migratory activity in caged migrants) were slightly lower than those observed at the same hour of night in which *Zugunruhe* did not occur. On the other hand, Bergman (6) has reported that the development of *Zugunruhe* in caged thrushes was accompanied by an elevation of body temperature to the extent of about 0.5°C. In each of the studies cited above, the data obtained were neither extensive nor were they analyzed statistically. It is well known (7, 8) that the struggling incident to the handling of a bird is accompanied by an increase in body temperature. Udvardy (8) has found the difference between the mean temperature at initial handling and the mean standard temperature in several species to be frequently in the order of 0.5–1.0°C. Although these differences are certainly in part the result of the muscular activity of struggling, doubtless other factors are involved; therefore it would be precarious to regard them as an indication of the order of change in body temperature which might occur with the activity of flight.

Through the kindness of Mr. William Dawbin it was possible to accompany an expedition from Victoria University College, Wellington, New Zealand, in conjunction with his investigations on the tuatara, *Sphenodon punctatus*. This expedition spent a week (late November–early December) in the outer Cook Strait, primarily on Stephens and Trios Islands. These islands have large breeding colonies of petrels of which the fairy prion, *Pachyptila turtur*, is the most common. Since our visit was during the breeding season, it was possible to obtain a fairly extensive series of data on the body temperatures of this species. These data have been the subject of a brief preliminary report (9).

PROCUREMENT OF DATA

Prions were procured under several different conditions for purposes of this study. From late evening until well after midnight large numbers were in flight over the islands. Many of these were returning from sea; however, quite probably they were joined by birds which had spent the day in the burrows. Many flew sufficiently close to the ground to allow us to catch them from flight with insect nets. Because of the large numbers which were alighting it was also possible to capture on the ground a substantial number within one minute after flight. Since a subsequent analysis of the data revealed no differences between the cloacal temperatures of those taken directly from flight and those taken immediately after alighting these were combined as *group 1* (table 1).

It was also possible at night to obtain with a net a considerable number of birds which were at rest or walking about on the surface of the ground. Some of these doubtless had been in flight earlier although not within several minutes of the time of capture. We also removed calling birds from the nesting burrows. Again some of these may have been in flight earlier although

Received for publication June 27, 1955.

[1] The data on which this paper is based were obtained while the author was a Fulbright Research Scholar at the University of Otago, Dunedin, New Zealand. The author wishes to acknowledge the assistance of Dr. C. A. Fleming in the procurement of the data.

not within a few minutes of capture. Since no differences were found in cloacal temperature between those birds taken from the ground and the calling birds taken from the burrows, the two groups have been combined into *group 2* (table 1).

During the day we obtained prions from the nesting burrows; most of these were incubating but none had young. Hatching dates for this species on Whero Island, south of New Zealand, are from mid-December to early January (10). Since there were no apparent differences between birds from burrows with eggs and those without, these were combined in *group 3* (table 1).

Although there may well be an advantage in obtaining birds by shooting in order to avert the thermogenic effect of struggling (11, 12), such was not possible under the conditions of this investigation. All birds were handled in such a manner as to reduce struggling to a minimum.

The weights of six randomly selected birds ranged from 114 to 130 gm.

Cloacal temperatures were measured with previously calibrated thermometers. Bearing in mind the differences in temperature which may exist in different parts of the body (13), care was exercised, as far as possible, to insert the thermometer precisely 2 cm into the cloaca. Readings were made 1 minute after insertion. It had been established in the laboratory earlier that the thermometers initially at 20°C would record temperatures of the order of 40°C within 0.05° in about 30 seconds. Between measurements the thermometer was kept warm in the hand of the operator. The time elapsed between capture and the first temperature recording was never more than 2 minutes. The data obtained in this way are summarized in table 1.

In order to ascertain the effect on cloacal temperature of holding the birds, in a number of cases temperatures were recorded at intervals of approximately 2 minutes after the initial readings. The data obtained are summarized in table 2. Although it would appear from the mean fluctuations that there may have been an upward trend, individual variations were actually quite random. Statistical analyses give no basis for the assumption of such an upward trend.

TABLE 1. CLOACAL TEMPERATURES IN FAIRY PRIONS

Group	Description	No.	Cloacal Temp. °C			
			Mean*	σ	Max.	Min.
1	Taken in flight or within 1 min. after flight	72	41.5	0.21	42.7	40.6
2	Active birds taken on ground or from burrows at night	28	39.9	0.54	41.2	38.1
	Incubating birds taken from burrows during the day	43	38.6	0.46	39.9	37.1

* The differences between the means for *groups 1* and *2*, *2* and *3*, and *1* and *3* are significant ($P < 1\%$).

TABLE 2. CLOACAL TEMPERATURES IN FAIRY PRIONS; FLUCTUATIONS AFTER INITIAL MEASUREMENT*

Group	Interval in Min.	No. of Birds	Mean Fluctuation °C
2	2	14	+0.1
	4–5	14	+0.1
	6–7	11	+0.1
3	2	10	+0.3
	4–5	7	+0.3
	6–7	7	+0.3

* Initial measurement was made within 2 min. after capture.

DISCUSSION

It is of interest to compare the results of this investigation with the data obtained by Folk (14, 15) on another small petrel, *Oceanodroma leucorhoa*. Mean standard body temperature, obtained in the laboratory, was 39.1°C; mean maximum body temperature evoked by muscular exercise was 39.4°C. A much lower mean, 37.2°C, was reported for incubating birds during the day. Ten adults with a mean temperature of 37.0°C in the burrow showed an increase to mean 39.7°C with forced exercise. The mean body temperature of another small petrel, *Oceanites oceanicus*, has been recorded by Roberts (16) as 38.8°C without reference to time of day or state of activity. Most of the data available in the literature (17–21) concerning other species of petrels, including the albatrosses, indicate body temperatures generally of the order of 39–40°C.

With respect to mean temperatures of the three groups of fairy prions, it cannot be contended, because of the known lability of avian body temperatures, that the means recorded in table 1 are precisely characteristic of flying birds, active nonflying birds and incubating birds respectively. However, in view of the random fluctuations obtained in handling and magnitudes of the mean fluctuations, it appears reasonable to conclude that the differences among the means, which are statistically significant (table 1), represent characteristic differences among these states of activity.

SUMMARY

Cloacal temperatures obtained from 143 fairy prions, a small species of petrel, indicate that body temperature in flight is about 1.6°C

higher than that of an active bird on the ground and about 2.9°C higher than that of an incubating bird during daytime.

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Relationship of Age and Sex to Early Mixing of Na^{24} in Normal Man[1]

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THE RATE at which various gases are eliminated from the human body has been shown to be slowed by the aging process (1–3). The processes of diffusion and blood circulation are important controling factors of the rate of gas elimination through the lungs. Since diffusion seems to be very rapid and with similar rate constants for various gases (2), the limiting factor in the rate of elimination has been assumed to be the blood perfusion of the body constituent tissues (2).

In previous human studies (4) with $Na^{24}Cl$ it was observed that an early component of sodium movement was much faster in younger than in older men. The early component observed subsequent to a single intravenous injection of Na^{24} is of the same order of magnitude as one of the rates of blood mixing. Since the blood-mixing process is closely related to blood circulation, it was thought that these measurements could provide a better method of determining the relationship of blood circulation to aging.

The individual variations observed in normal subjects of the same age suggested that these measurements might also be useful as a test for determining the physiological age vs. the chronological age of human beings, using as an aging criterion the effect on blood circulation.

The aim of the present work was to measure an early rate of Na^{24} movement on a larger number of normal humans of both sexes to evaluate such variables as regression slope of an early mixing rate with age, the correlation coefficient between these two variables for a large part of the life span, the existence of any sex difference, the individual variations and the error of measurements. The knowledge of these variables is considered important not only for judging these measurements as a test of physiological age and the aging process but also for studying the effect that physical, chemical and pathological factors might have on the dynamics of molecular transportation in humans.

Received for publication March 14, 1955.

[1] This work was supported in part by the National Heart Institute, National Institutes of Health, and in part by United States Atomic Energy Commission.

METHODS

Subjects. The subjects in these studies were normal men and women from 22 to 90 years of age who volunteered for the measurements. They were apparently in good health with no past history of serious diseases. They were working or retired persons whose living and working conditions indicated that they were a sample of the average population. Sixty-three men and 70 women with ages evenly distributed within the age range chosen were studied. There were approximately five subjects in each 5-year class interval of the span of age.

Technique. Subjects rested in the supine position for at least 15 minutes before injection. An isotonic solution of 30–50 μc of $Na^{24}Cl$ was then injected in a few seconds into an arm vein. A scintillation counter completely shielded with about a 2-inch thickness of lead and a frontal aperture of $\frac{1}{4}$ inch was placed over the precordial region, about 5 cm from the mid-line at the fourth intercostal space. The scintillation counter was connected to a scaler and a recording device which plotted the accumulated counts for each 24-second interval. These counts were expressed on the record as the counting rate per minute. Measurements were continued from a minimum of 35 minutes in a few cases to 2 hours in others.

Analysis of Data. The concentration of radiosodium measured over the heart region, following the intravenous injection of $Na^{24}Cl$, increases very rapidly to a maximum 15–30 seconds after administration. It is followed by a fast decrease during the first minutes, continuing with a slower disappearance thereafter.

From previous analyses of Na^{24} time-concentration curves in normal human beings (4) at least six components were determined by using the regular graphic method of estimating exponential functions. Of these components, the one designated as the second was considered of particular interest in the present studies.

The second component is practically resolved within the first 90 seconds to 10 minutes in adults. Therefore, in most curves from measurements continued from 35

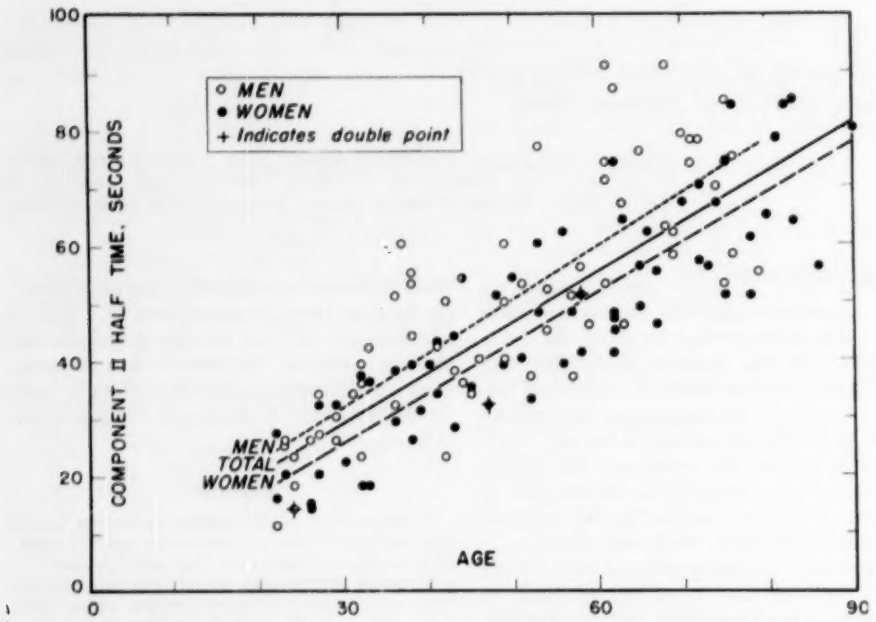

FIG. 1. Relationship of age of subjects to half-time of second component as determined over heart region of normal subjects of both sexes. Regression lines obtained from 63 men, 70 women and the 133 subjects of both sexes.

minutes to 2 hours, the first three components, plus the largest part of the fourth, are included. Estimations of the fifth and sixth components were not possible in the present curves because of the short time of measurement. Consequently, approximate values of the fifth and sixth components had to be calculated. The hypothetical fifth component was drawn on semilogarithmic paper beneath the actual curve as an almost horizontal line for the first 35 minutes. For the fifth component the amplitude at zero time was assumed to be 10% of the concentration of the curve up to the fourth component, the rate having a half-time of over 1 hour. These were approximate values obtained from previous longer measurements with sodium. The hypothetical fifth component line was then subtracted from the actual curve and the fourth, third and second components were extracted following the regular graphic method for estimating exponential functions. Because of the slowness of the fifth and sixth components in relation to the second, plus the small amplitude of the fifth, the use of an average value as a correction factor to subtract the effect of these slow components adds only a negligible error to the estimation of the second component.

When measurements are continued for at least 90 minutes a simpler procedure for estimating the rate of the second component can be followed by extrapolating either the fourth or the fifth components from the actual curve to zero time. The subsequent extraction of the earlier components will result in a half-time of the second component with slightly larger error than with the method previously described.

The second component is expressed in terms of half-time in seconds instead of the constant K because of the more common usage of this value.

Statistical Analysis. The correlation chosen to be analyzed was *a*) between the half-time of the second component and the age of the subject and *b*) between the values of two half-times obtained by either separate measurements on the same subject or two evaluations of the same measurement. For that purpose, in both cases the method of least squares was used to determine the regression and correlation coefficients and the standard error of estimate.

RESULTS

The analysis of the curves from the group of subjects studied indicates that the second component develops during the first 1.5 minutes for the faster rate subjects and during 10 minutes for the slowest; the half-time ranged from 11 to 91 seconds. The half-time of the second component was plotted against the age of the subjects as shown in figure 1. Although a wide scattering and overlapping is observed for the individual points of men and women, the general trend is for the rates to be slowed

with aging of the subjects. In general, faster rates were observed in women than in men of the same age, as shown in the regression lines of both sexes. The regression line was assumed to be a straight line. The error of individual measurements is too large and the number of subjects too small to prove whether there is a nonlinear regression.

The results and statistical analysis of this group are also shown in table 1. As may be observed, the average half-time for the second component in men was 50.5 seconds for an average age of 49.8 years, whereas in women it was 45.6 seconds for an average age of 53.1 years. The regression coefficient, b_{ha}, was for men 0.93 ± 0.06 sec/yr., for women 0.86 ± 0.03 sec/yr. and for the group of both sexes 0.87 ± 0.04 sec/yr. The standard error of estimate, σ_{ha}, was 11.9 seconds for men, 8.8 seconds for women and 11.1 seconds for the group including both sexes. The correlation coefficient, r_{ha}, was 0.79 for men, 0.88 for women and 0.65 for both sexes together.

To estimate the difference between the second component half-time of both sexes, 50 years was chosen as the age for comparison, since the average age of both groups was different. The half-time corresponding to 50 years estimated from the regression line is 50.7 seconds for men and 42.9 seconds for women, or a difference of 7.8 seconds (table 2).

To determine the possibility of the results being influenced by knowing the age and sex of the subjects, the time-concentration curves of 18 men and 19 women from 30 to 50 years of age were replotted, coded and shuffled to be estimated again by the same person without knowledge of the age and sex of the individual. In addition, nine curves taken at random from the same group of subjects were duplicated and mixed with the others. This group of subjects, hereinafter referred to as the unknown group, was compared to the same subjects, hereinafter referred to as the known group, which had previously been estimated with knowledge of their age and sex.

The results between these known and unknown evaluations are presented, part in table 2 to compare the sex difference, and part in table 3 to compare the regression and correlation coefficients of the half-time with age and the standard error of estimate.

TABLE 1. RELATIONSHIP OF AGE TO RATE OF SECOND COMPONENT

Subj.	No.	Av. Age yr.	T½ sec.	σ_{ha} sec.	b_{ha} sec/yr.	r_{ha}
Men	63	49.8	50.5	11.9	0.93±0.06	.79
Women	70	53.1	45.6	8.8	0.86±0.03	.88
Mixed	133	51.5	47.6	11.1	0.87±0.04	.65

Average half-time T ½ of second component. Regression coefficient b_{ha} standard error of estimate σ_{ha} and correlation coefficient r_{ha} of half-time with age in group of 133 normal subjects of both sexes.

The reliability of two tests was estimated by measuring 20 subjects twice on different days and each measurement was estimated only once. The average half-time was 47.6 seconds for the first measurement and 45.4 seconds for the second measurement. The standard error of estimate, σ_{tt}, was 8.4 seconds and the correlation coefficient, r_{tt}, between both tests was 0.81.

The reliability of two separate evaluations of the same measurements was determined by comparing the estimation of 37 known subjects with the estimation of the same subjects from unknown curves. The average half-time was 39.0 seconds for the first evaluation and 38.8 seconds for the second. The standard error of estimate, σ_{tt}, was 8.0 seconds and the correlation coefficient, r_{tt}, between both evaluations was 0.75.

In a group of nine unknown subjects where two evaluations from the same curve were made, the average half-time for the first evaluation was 39.6 seconds and 40.8 seconds for the second. The standard error of the estimate, σ_{tt}, was 6.4 seconds with a correlation coefficient, r_{tt}, between both evaluations of 0.88.

The average of two measurements of the same individual with one evaluation each, gave an r_{ha} of 0.77 and a σ_{ha} of 8.7 seconds, whereas for the average of two evaluations of the same curve r_{ha} was 0.58 and σ_{ha} was 8.6 seconds.

Since measurements were performed at various times, the data were divided into subgroups of morning and afternoon, and regression lines were estimated in both sexes, in order to determine whether there was any difference. For both groups, as shown in figure

TABLE 2. STATISTICAL SIGNIFICANCE OF SEX DIFFERENCE OBSERVED IN HALF-TIME OF SECOND COMPONENT

Subj.	Age Level	T ½ sec.		*t* Ratio	Probability
		M	W		
Known, 133	50	50.7	42.9	3.84	0.01–0.001
Known, 37	40	42.6	35.7	2.55	0.02–0.01
Unknown, 37	40	42.2	35.6	2.00	0.06–0.05

First group, 63 men and 70 women whose age and sex were known. Second and third group formed by same 18 men and 19 women, 30–50 yr. old, evaluated with knowledge of their age and sex in first group, and from unknown curves in the second. The *t* ratio and probability for each group.

2, the regression lines indicate faster half-times in the morning than in the afternoon in women aged from 22 to 55 years, in men aged from 22 to 65 years and slightly the opposite in the later years for both men and women. The regression slope was increased per year of life in the morning group to 1.02 ± 0.14 seconds in men and 0.94 ± 0.10 seconds in women, and in the afternoon group to 0.84 ± 0.06 seconds in men and 0.82 ± 0.09 seconds in women. However, the number of subjects studied was too small to give statistical significance to this difference.

DISCUSSION

From the various components previously observed in the sodium concentration curves (4), the second component was selected because it shows a marked association with the aging process and is more accurately estimated in a shorter time than the other components.

The correlation coefficient between the age of the subject and the rate of the second component was quite high and can be considered statistically significant. The correlation coefficient was further improved when each sex was estimated in a separate group.

The significance of these results is somewhat limited by the fact that the age and sex of the subjects were known to the investigator who analyzed the data. Therefore, it was thought necessary to estimate again some of the same subjects whose age and sex would be unknown to the person estimating it. In general, the results of this control evaluation show very slight differences between both estimations. The standard error of estimate, which indicates the data spread, was larger for the unknown group of men and women than for the known group by 1.8 seconds. This can be interpreted as a very small influence in knowing the age and sex of the subject. However, the differences obtained in both calculations are too small to change the significance of the age and sex relationship observed in the large group of known subjects.

The correlation between age and rate of the second component was more significant in the group of 133 subjects than in the group of 37 subjects, being in the known group slightly better than in the unknown. This difference of better correlation for the large group could be explained in part by the fact that the age of the subjects ranged from 22 to 90 years, whereas in the small group of 37, the ages ranged from 30 to 50 years.

The sex difference observed in all groups was quite marked, as summarized in table 2. In the large group of 133 where the sex was known, the difference was statistically very significant. The probability that the sex difference in that group is due to sampling is of the order of less than 1% with a *t* ratio of 3.84. In the small group of 37 where the sex was known, the same probability is between 1% and 2%, with a *t* ratio of 2.55. In the same small group of subjects where the sex was unknown, the probability is between 5% to 6% with a *t* ratio of 2.00. The sex difference in the latter group should be statistically considered as only fairly significant. The decrease in significance of the small group might be explained as due to the smaller number of subjects, and also that the average age was 40 years instead of 50 years for the larger group, and the sex difference seems to increase with age.

The differences observed in the regression coefficient b_{ta} in men and women, which could also be expressed as representing the aging rate of their circulatory system, are statistically less significant than the sex difference observed in the average half-time for the same subjects. The difference in the regression coefficients in both sexes gave a *t* ratio of 1.81 with a probability of 0.10 to 0.05.

Sex differences have been found in dynamic

studies of gases in humans. The rate of carbon monoxide elimination at rest was found to be faster in women than in men of approximate age (5). This sex difference was approximately maintained when the CO elimination rate was accelerated by exposure to an ambient pO_2 from 0.2 to 2.5 atmospheres. The oxygen recovery rate after mild exercise was found to be slower in a group of women than in men (6), although the oxygen recovery rate has been shown to be influenced by the aging of the subjects (1). Differences in physical fitness in both sexes might explain this reverse situation. The blood velocity rate has been determined as slightly faster in women than in men (7). The standard error of estimate σ_{ha} from the regression line is a good index to estimate the significance of differences from normal behavior that might be observed in one or more measurements. For a single measurement, the standard error is quite large; it can be decreased by separation of sex, morning and afternoon measurements, as well as by two independent evaluations of the same curve. The average of two measurements on the same individual on different days also decreases the standard error.

The four main sources of variations contributing to the standard deviation are: *a*) the technical errors, *b*) the error in the graphic evaluation of exponential functions, *c*) the individual variations and *d*) the physiological variations. The number of cases analyzed is not large enough to evaluate all these individual variables statistically. However, it seems that the method used for estimating the exponential functions is the largest source of individual error. Apparently the individual differences and physiological variations under similar conditions of measurement are not very large and the effect of the aging process in itself seems to be the most outstanding phenomenon of these observations.

Interpretation of the Second Component. Substances introduced into the blood stream are transported within the blood and sometimes to other compartments following several rates of movements. The interrelation between the rates determined from the experimental data and the rates of mixing and penetration in the various compartments are not well established as yet, and are to be discussed in more detail elsewhere (8). It can be stated here, however, that the mixing of a substance within the blood after the intravenous administration of a single dose occurs primarily following three components.

TABLE 3. COMPARISON BETWEEN TWO SEPARATE EVALUATIONS FROM SAME CURVES OF SAME SUBJECTS

b_{ha}, sec/yr.			σ_{ha}, sec.			r_{ha}		
M	W	Mixed	M	W	Mixed	M	W	Mixed
Known subjects								
0.72	1.12	0.91	8.2	7.8	8.7	.46	.66	.55
Unknown subjects								
0.77	1.16	0.96	9.8	9.7	10.5	.45	.60	.48

First evaluation, labeled as 'known,' is from subjects whose age and sex were known. Second evaluation of same subjects from plotted curves with no clue to subject's age and sex is labeled as 'unknown.' Regression coefficient b_{ha} of half-time T $\frac{1}{2}$ of second component with age, standard error of estimate σ_{ha} and correlation coefficient r_{ha} of group formed by 18 men and 19 women, 30–50 yr. old.

The first component represents the mixing of the injected substance within the lung pool and distribution to all the other body pools through the arterial tree. The second component probably represents the contribution to the mixing by the pools of high perfusion rate and situated close to the heart. The third component would represent the contribution to the molecular mixing by the pools of low perfusion rate and situated farther from the heart.

The rate of the second component, as measured after the sodium administration, seems to be of the same order of magnitude as similar early components observed after the intravenous administration of substances nondiffusible into the interstitial fluid (9). The diffusion of Na^{24} into the interstitial fluid through the capillary walls apparently does not modify the rate of movement of the second component. The second component in the sodium measurements is interpreted here as one of the rates which develops during the blood mixing.

What mechanism during the aging process might be responsible for producing such marked slowness of the rate of the second component is an interesting question to be elucidated. The possibility that capillary permeability to sodium might decrease during the aging process is difficult to prove. The present knowledge of dynamics of substances non-

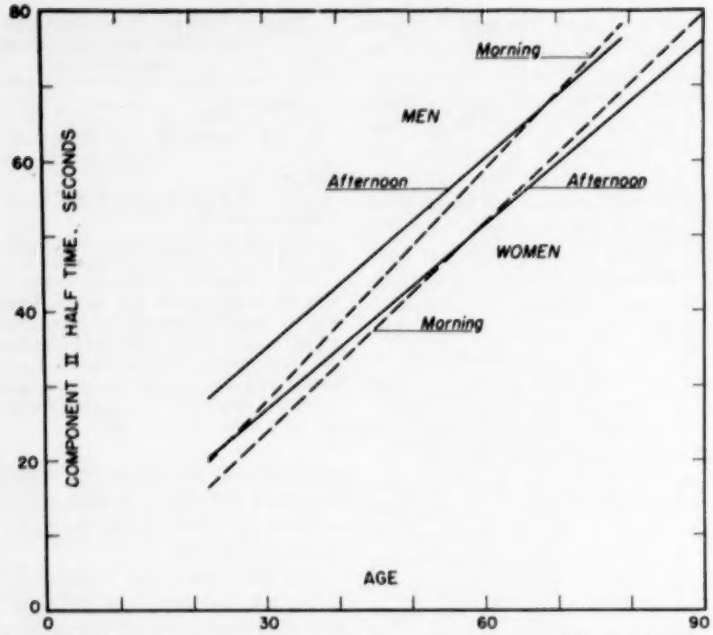

FIG. 2. Relationship of age of subjects to half-time of second component and time of day of performance of measurement. Regression lines obtained from groups of normal individuals. Men, 26 in morning and 37 in afternoon; women, 24 in morning and 46 in afternoon.

diffusible into the interstitial fluid (9) shows slight differences with no statistical significance. The hypothesis of a decrease in capillary permeability with aging has been controversial. The results obtained by Holman (10) in dogs using large molecules like proteins do not agree with a previous suggestion of a decrease in permeability by Drinker (11). The less restricted diffusion through the capillary wall for sodium chloride than for nonelectrolyte molecules, as shown by Pappenheimer *et al.* (12), decreases the probability that aging may influence the permeability of capillaries to sodium.

If capillary permeability is not an important factor in slowing the second component, the effect of aging in the cardiovascular system and consequently in hemodynamics should then be assumed to be the most important factor. Among the anatomical and physiological changes in the cardiovascular system observed during the aging process should be mentioned a decrease in cardiac output (13), an increase in the sclerosis of the arteries, peripheral resistance, blood pressure and blood viscosity and an acceleration in the pulse wave. The blood flow of certain organs, such as the kidneys (14) and muscles (2) has been reported to decrease markedly with age. Of all the factors enumerated, the increase in arterial sclerosis and peripheral resistance in the aging of normal human beings seem to be the factors more likely to influence the remarkable slowing of the rate of the second component.

SUMMARY

The measurement and evaluation of an early rate of Na^{24} movement in normal subjects of both sexes is described. The rate of Na^{24} movement designated as the second component is markedly slowed during the aging process, and the correlation between the rate and age is significant. The rate of the second component is more rapid in women than in men of the same age. The sex difference observed for the average half-time of the second component at the chosen age of 50 years is statistically very significant. The differences observed in the

regression coefficients of both sexes are, however, of less statistical significance.

A single measurement and evaluation of the half-time of the second component has a limited value for determining the relationship between the rate of blood mixing of an individual to his chronological age because of the large standard error. More than one measurement and evaluation as well as technical improvements are necessary to decrease this error. The present technique is adequate to study, in a group of subjects, the deviations from the normal regression line of the movement rate versus age.

The authors wish to acknowledge their appreciation to Mrs. Cathrine Cohn for her aid in securing persons for these studies.

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Diurnal Variation in Rate of Alcohol Metabolism

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DIURNAL variation of various metabolic processes and the results of such changes have been the object of investigation by many physiologists over a long period, and the literature on the subject is voluminous. Diurnal variation in temperature of the body and changes in the normal rhythm were commented upon by Foster (1). More recently diurnal variations of water, electrolytes and creatine excretion (2), hepatic metabolism as measured by glycogen content (3), blood cellular components (4) and other metabolic processes have been studied. Our excuse for presenting yet another study along these lines is twofold. First, the metabolism of alcohol lends itself readily to study, since this substance is metabolized at an essentially constant rate regardless of its concentration in the body (5), and the initial metabolic process takes place to a significant degree in the liver alone (6), while its concentration in body fluids is subject to accurate measurement. Second, recent work (7) has appeared which casts doubt on the previously held view that the rate of alcohol metabolism remains relatively constant in a given individual from time to time, a discrepancy which might be explainable on the basis of diurnal variation.

METHODS

Six human subjects were used. Alcohol was administered by mouth in the form of whiskey diluted with water to a final concentration of approximately 20% alcohol by volume. The objective was, by the repeated ingestion of small doses of alcohol, to achieve a slowly rising concentration in the body, the rise being slow enough so that an experiment could be continued for as long as 48 hours without the onset of severe degrees of intoxication. The amount of alcohol required for each subject was estimated by giving a test dose of 1 cc/kg body weight, after which blood or saliva alcohol concentration was determined hourly by a micro-diffusion method (8). By extrapolation of the curve of blood alcohol against time, the period required for the metabolism of this amount of alcohol could be estimated, and thus the amount metabolized per hour. This dose was increased by 10 or 20%, and was effective in producing the desired slowly rising concentration.

Each subject was studied over at least a 36-hour period on at least two occasions. The predetermined dose of alcohol was given hourly except that, in order to allow the subject a more restful night, the 1 A.M. and 2 A.M. doses were combined with the midnight dose, and the 4 A.M. and 5 A.M. doses with the 3 A.M. dose. It has been adequately demonstrated (9) that the higher alcohol concentration thus achieved would not produce any change in rate of alcohol metabolism. Specimens of either blood or saliva were collected immediately prior to the administration of alcohol, and thus an hour after administration of the preceding dose, by which time absorption would be reasonably complete. The subjects took a regular diet, at approximately 7 A.M., 12 M. and 6 P.M.

RESULTS

Figure 1 shows a graph of the alcohol concentration of *subject JL* over a 48-hour period on an hourly dose of 17.5 cc of 95% alcohol. It is clear that in this subject from about 8 P.M. until 10 A.M. the alcohol curve rose, indicating an excess of intake over metabolism, while during the period from 10 A.M. to 8 P.M. the curve fell, due to an increased rate of metabolism. This was true to a varying degree in all five subjects on a normal diurnal regimen, sleeping at night. A series of curves of increment or decrement of salivary or blood alcohol level over the previous level was plotted against the time of day for each experiment. Figure 2 is a graph of the increment or decrement of alcohol levels in the cases studied. Figure 3 is a composite 24-hour curve of the means at any time, derived from the individual curves, and shows very strikingly the diurnal variation. A sixth subject was a nurse on night duty; in her case the rhythm was reversed on three separate studies, in spite of the fact that the experimental period was over her day off, when she chose to sleep at night. This last is only

Received for publication March 8, 1954.

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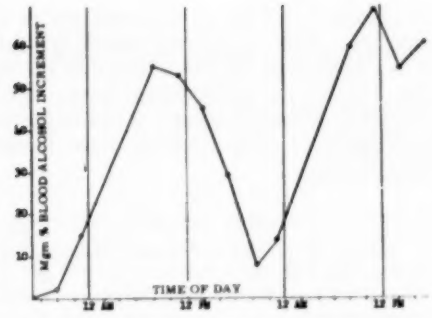

FIG. 1. Blood alcohol levels in mg % are presented for *subject JL* on an hourly dose of 17.5 cc of alcohol over a period of 48 hr.

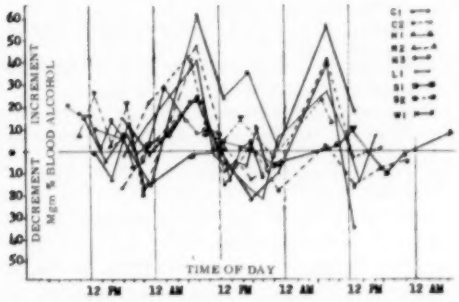

FIG. 2. Increment or decrement in mg % of blood alcohol in 9 studies of more than 24 hours' duration each. Previous blood alcohol level is represented by horizontal line marked by an asterisk (*). The 9 cases are identified by the symbols in the figure.

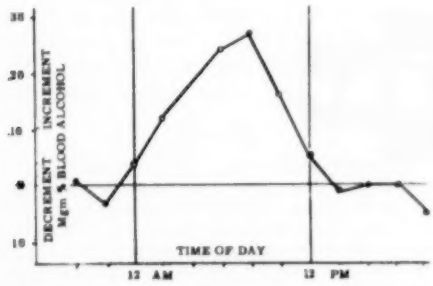

FIG. 3. Mean increment or decrement in mg % of blood alcohol levels in the 9 cases from fig. 2.

mentioned parenthetically, but is interesting since diurnal rhythms largely are associated with the development of habit patterns of sleep rather than specific sleep episodes.

DISCUSSION

The foregoing data show quite clearly that the rate of disappearance of alcohol from the body does not proceed at a constant rate, but varies with the time of day in a pattern determined by the sleep habits of the individual. The degree of this variation is different from one individual to the next, and can amount to as much as 25% of the hourly rate of metabolism of alcohol. This at once eliminates the possibility of changes in excretion of alcohol in the breath and urine accounting for the phenomenon, since elimination by these channels has been shown to account for less than 10% of ingested alcohol, and with the low concentrations prevailing in our subjects it would be far less. Neither can variability in rate of absorption at different times of the day be invoked, since the duration of the upward or downward trend is far too long to be explicable on the basis of alcohol retained in the stomach. It would seem inescapable, therefore, that the actual rate of combustion of alcohol varies with the time of day. The correlation of the diurnal variation of body temperature with that of alcohol metabolism would appear to be close. This is not surprising as the body temperature is but a reflection of the metabolic rate and the rate of heat loss, both of which are depressed during sleep. The independence of the rate of alcohol metabolism from actual sleep is interesting, however, as the rise in blood alcohol levels starts well before the normal time of retiring, and the morning fall not until after breakfast. The possible relationship to the intake of food is more intriguing, since the time of rapid metabolism corresponds fairly well with the part of the 24 hours in which our subjects took their meals. It has been demonstrated (9) that fasting reduces the rate of alcohol metabolism, while certain amino acids certainly increase it, and the degree of such change is of the same order as we found. Whether this effect of food on alcohol metabolism is the sole cause of the diurnal variation demonstrated awaits the test of further experimentation with precise control of food intake.

Practically, the main point of interest lies in the obviously important factor of the time of day chosen to study metabolic functions of this sort. In the case of alcohol, some effort has

been made to use it as a test substance in the study of liver function (10). Certainly the time of day the test was made would have a profound influence on the result. Equally, any comparison of rate of alcohol metabolism in experimental work, either between different subjects or the same subject at different times, must take into account the diurnal variation we have demonstrated.

From the point of view of the user of alcohol, it is interesting to point out that the 'one for the road' taken late at night might be expected to produce a more lasting rise in blood alcohol concentration, due to slower metabolism, than a drink of the same size at the cocktail hour.

SUMMARY

Evidence is presented of a diurnal variation in the rate of metabolism of ethyl alcohol in man, based on the level of alcohol in the body fluids of six subjects while ingesting hourly doses of alcohol over an extended period. This variation in rate of metabolism is not due to changes in excretion of alcohol nor in its absorption from the gastrointestinal tract. It is not predicated on the actual time of day but rather on the habitual sleep pattern of the individual. Whether or not it is due in part or in whole to ingestion of food awaits further investigation.

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Uropepsin Response to Cortisone in the Aged[1]

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AN INCREASE in uropepsin excretion following administration of ACTH or cortisone[2] has been reported from various laboratories (for references see (1)). In this paper we wish to report observations in aged subjects who showed considerable variations in uropepsin values during periods of administration of cortisone. Preliminary results have been stated previously (1).

Six female subjects from the Drexel Home for the Aged, between 72 and 82 years of age, were used. Five of them were suffering from osteoarthritis and were chosen because they were to be treated with cortisone. Except in *subject S*, the arthritis was of slight to medium degree. *Subject S* was suffering from severe arthritis in her fingers, both wrists and knees; 20 years ago she underwent subtotal thyroidectomy for toxic goiter. All subjects had some degree of arteriosclerosis. *Subject B* had a healed duodenal ulcer which had been quiescent for the last 2 years, but she had had several periods of ulcer hemorrhage before. *Subject He* was obese, but otherwise normal. None of the subjects had an elevated body temperature or leucocytosis, all had red blood cell counts and hemoglobin values within the limits of normal, and none had anemia of any kind or was suffering from diarrhea, vitamin or nutritional deficiencies.

METHODS

In all subjects, three or more control determinations of uropepsin were performed before and after the administration of cortisone, except in *subjects H* and *S*, in whom controls were done only before cortisone.

Twelve-hour night urines were collected under toluene and no food or drink was taken during that period. One collection period was performed with the use of indwelling catheters in four patients in order to check urine volumes. The urines were kept refrigerated and uropepsin determinations were performed within 24 hours after collection. Uropepsin was determined on nondialyzed urine (2) by a modification of the method of Anson and Mirsky (3), and a unit of uropepsin is defined as that amount of enzyme which releases 1 mEq $\times$ 10^{-4} of 'tyrosine' from a hemoglobin substrate in a 10-minute incubation period at 37°C (4). Uropepsin values are reported as units excreted per hour. Cortisone administration was started with 50 mg/day, except in *subject S* (100 mg) and it was ended with gradual tapering off of the daily dose.

Since it was not possible to perform gastric secretion tests with intubation in these aged subjects, tubeless gastric analysis was performed according to the procedure of Segal, Miller and Plumb (5). Squibb carboxylic resin indicator compound with azure A was employed, and gastric secretion was stimulated with caffeine.[3]

RESULTS

The control values for uropepsin varied considerably in all six subjects. We have previously determined uropepsin levels in a large number of normal subjects in order to establish standard values for age and sex. According to our results, the mean value for the present female group, all above 60 years of age, is 98 $\pm$ 9 units, with a variation between 6 and 316 units (1).

In five of the six subjects, no elevation of uropepsin values beyond the control periods was observed, and the variations of uropepsin values during administration of cortisone were as considerable as during the control periods. The duration of cortisone administration was, in the order of the subjects in table 1: 5, 19, 19, 55, 20 and 18 days. An elevation of uropepsin values during administration of cortisone occurred only in *subject B*, who had a history of bleeding duodenal ulcer. However, no signs of activation of the ulcer were observed during this period or later, although the patient was observed carefully. Elevation of

Received for publication September 6, 1955.

[1] Supported by the American Cancer Society, Illinois Division, and by a grant from Mr. John Gottlieb for studies in old age. The department is in part supported by the Michael Reese Research Foundation.

[2] Cortisone supplied by Dr. C. J. Donovan of The Upjohn Company.

[3] Supplied by Dr. M. I. Hewitt, Squibb Institute for Medical Research.

TABLE 1. UROPEPSIN IN FEMALES TREATED WITH CORTISONE

Date 1955	H., 82 yrs., 140 lbs. Arthr. Art. scler.		B., 72 yrs., 135 lbs. Arthr. Inact. duod. ulc. Prev. hemorrh.		L., 81 yrs., 128 lbs. Arthr. Dyspeps, low gast. bas. secr.		S., 78 yrs., 171 lbs. Arthr. Thyroidect. 20 yrs. Obese, edema w. Cortis.		Be., 80 yrs., 188 lbs. Arthritis. Obese		He., 80 yrs., 195 lbs. Obese	
	Uropep.	Cortis. mg p. d.	Uropep.	Cortis. mg p. d.	Uropep.	Cortis. mg p. d.	Uropep.	Cortis. mg p. d.	Uropep.	Cortis. mg p. d.	Uropep.	Cortis. mg p. d.
3-23	152	-	124	-	26	-	102	-	118	-	134	-
24	284	-	180	-	22	-	70	-	104	-	191	-
25	155	-	161	-	24	-	100	-	65	-	192	-
26								100				
27								100				
28		50		50		50		100		50		50
29	148	75	256	50	36	50	168	200	144	50	232	50
30	222	75	348	75	26	75	138	200	65	75	215	75
31		75		75		75		200		75		75
4-1	187	75		75	43	75	117	200	194	75	220	75
2				75		75		200		75		75
3				75		75		150		75		75
4			426	75	26	75	84	150	74	75	225	75
5			433	100	47	75	104	150	78	100	225	100
6			517	100	46	100	180	150	129	100	238	100
7				100		100		150		100		100
8				100		100		150		100		100
9				100		75		150		100		100
10				100		75		150		100		100
11			436	50	53	50	78	150	187	50	252	50
12			370	50	35	50	90	100	118	50	157	50
13				50		50		100		50		50
14				25		25		100		25		25
15				25		25		100		25		25
16				-		-		100		25		-
20			187	-	39	-	88	100	60	-	173	-
27			184	-	43	-	103	75	96	-	266	-
5-4					39*	-	166	75	68*	-	251	-
12							129*	50				-
19								50			194*	-

* By indwelling catheter.

uropepsin excretion was noted on the 2nd day of administration of 50 mg of cortisone, and it had returned to control values 5 days after cessation of cortisone administration. In *subject S*, who received the largest amount of cortisone over the longest period of time, not the least increase of uropepsin rates over the control values was observed at any time.

The results of tubeless gastric analysis performed on the last five subjects in table 1 indicated good secretion of free HCl except in *subject L*, in whom the test indicated achlorhydria. In *subject L*, the lowest values for uropepsin were found.

DISCUSSION

The considerable day by day variations in uropepsin excretion which we found in most of these cases indicate that positive changes in uropepsin output must be rather large in order to be significant.

The present observation that only one out of six aged subjects responded to administration of cortisone with an increased output of uropepsin is puzzling. None of our present subjects was suffering from any disease of the stomach or of the blood-forming organs that are known to depress or abolish uropepsin excretion, and all control values for uropepsin in our subjects were within the range of normal of the same age group, as stated above.

Since *subjects B, S, Be* and *He* secreted free acid according to the test employed, we must assume that the absence of increased uropepsin excretion during prolonged administration of cortisone was not due to a deficiency of gastric secretory mechanisms, as seen in pernicious anemia.

Our present results suggest that studies on uropepsin excretion with regard to the adrenal cortex should include multiple control tests, in order to be able to judge the wide variations that may occur normally. The possibility suggests itself that aged females do not, as a rule, respond to cortisone with increase in uropepsin production or uropepsin output in

the urine. The fact that one subject with a healed duodenal ulcer was the only one in six to show increased uropepsin levels while under cortisone treatment is of interest. No sign of activation of the ulcer was noted, although uropepsin rates increased from an average control value of 155 to as high as 517 units

SUMMARY

In aged female subjects, uropepsin excretion was estimated before, during and after administration of cortisone. During cortisone administration, elevation of uropepsin was observed in only one subject.

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SPECIAL COMMUNICATIONS

A New Type of Flow Resistor for Respiratory Studies[1]

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THE MOST widely used type of respiratory flowmeter is based on the principle that the pressure incident to laminar flow is a linear function of the volume flow (1). Early flowmeters of this type contained bundles of capillary tubes as the resistant elements. The use of Monel mesh wire screen for the flow resistor was introduced by Lilly (2) and by Silverman and Whittenberger (3), and most respiratory flowmeters now contain Monel screens. The calibration curves for such instruments are initially linear, and if a screen of sufficiently large area is chosen, a linear instrument of the desired capacity can be produced.

Two serious problems affect all such flowmeters, water-vapor condensation and mucous deposits in the resistor. If the Monel screen resistor is heated by a current passing through it, condensation is prevented. Obstruction by mucus is a greater problem since it may alter calibration significantly. The delicacy of mounting and cleaning these screens and the high cost of replacing them have led to a search for more rugged substances for resistors. Young (4) devised a rigid unit consisting of concentric cylinders. However, in instruments of this type, cylinders adequate to measure high flow rates must be inconveniently large.

DESCRIPTION OF APPARATUS

Resistor. We have found that porous vitrified abrasive wheels[3] may be used as satisfactory, rugged, and economical, flow resistors in a respiratory flowmeter (fig. 1).

Figure 2 shows the calibration curves for vitreous resistors of varying thicknesses. The ¼-inch thick, cup-shaped resistor has a linear response to 200 l/min. ± 4.2 l/min., and has been found the most satisfactory. In obtaining these curves, the Bernoulli principle was utilized, the inlet from which pressure was measured being placed so that it opened in the direction in which the stream of air flowed. This had the effect of extending the region of linearity.

The resistor has been used primarily for measuring the maximum rate of expiratory flow. No difficulty has ever arisen from collection of mucus. With the subject breathing quietly for more than 5 minutes, condensation of water vapor increases the pressure drop but during intermittent use at high flow rates this has been no problem. Blowing compressed air through the flow resistor satisfactorily returns the calibration to normal, and this should be done about once every 50 tests unless an interval of 24 hours has elapsed. The process of water-vapor condensation is quantitatively related to heat transmission by the object exposed to saturated air. Hence, to minimize condensation, a small object of low heat conductance and at a temperature close to 37°C should be used. In this apparatus, condensation is minimized by the use of a substance with low heat conductance and by the subject's grasping the metal mouthpiece so that it is heated to near body temperature. A shell heated to 37°C would obviate all such condensation and make the apparatus suitable for protracted use.

For cleaning, when indicated, a brush, and soap and alcohol were used, and after rinsing the

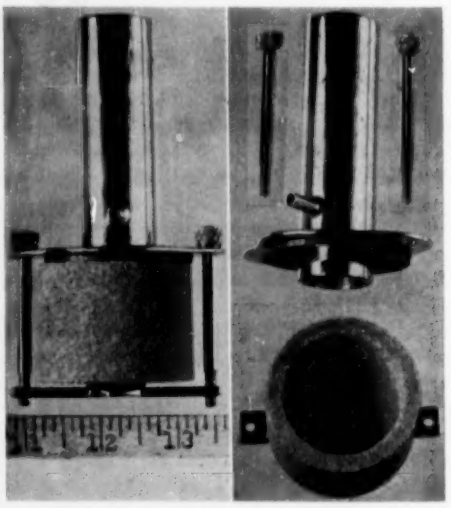


FIG. 1. *Left:* flow resistor using vitrified grinding wheel; *right:* exploded view.

Received for publication August 2, 1955.

[1] Aided by a Grant-in-Aid from the Subcommittee on Research Grants of the Council on Pharmacy and Chemistry of the American Medical Association.

[2] Present address: Dept. of Preventive Medicine, Harvard Medical School, Boston 15, Mass.

[3] Norton Co. no. 32A46-18VBE.

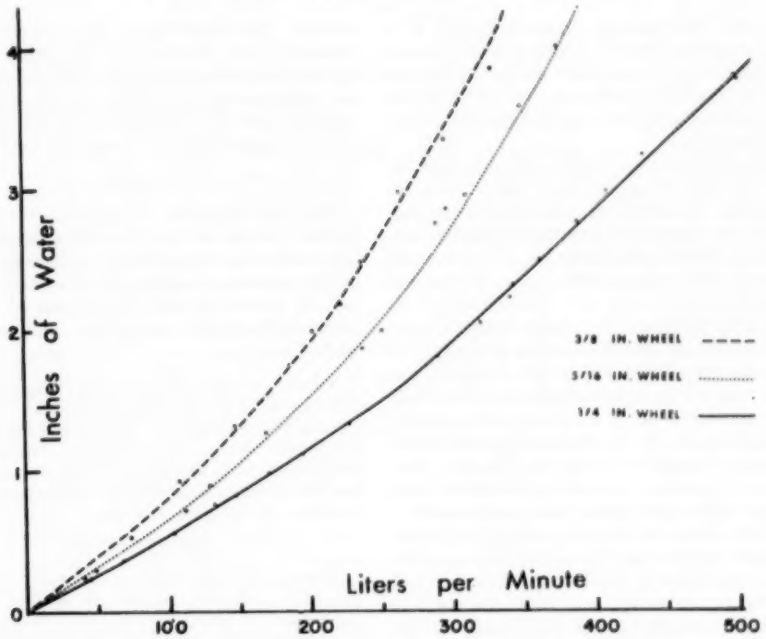
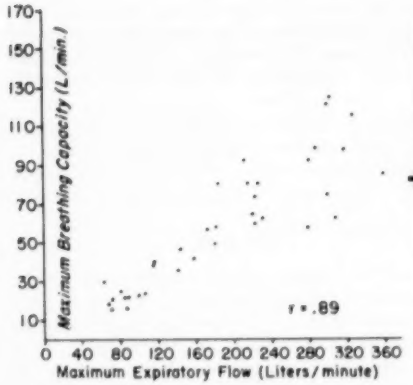


FIG. 2. Calibration curves of vitreous grinding wheels of varying thickness.



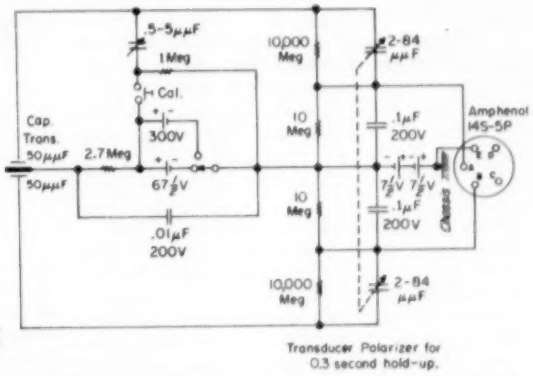


FIG. 3. *Left:* Scattergraph showing correlation between maximum expiratory flow test and maximum breathing capacity test.

FIG. 4. *Right:* Wiring diagram of capacitance transducer polarizer unit. Magnitude of pressure signal produces a shift of about 2 μμf. The 15-v. output bias is used to compensate for grid current in the EKG amplifier.

flow resistor was heat dried. The resistor was so rugged that no breakage occurred during a year's use, and the manufacturing uniformity is good enough that out of one lot of 22 wheels, 20 had essentially identical calibration curves. The initial cost was less than one dollar.

Flowmeter. Two pieces of apparatus have been developed for use with this type of resistor. The first is a very simple and compact unit for measuring maximum expiratory flow during a single forced expiration following maximum inspiration. The pressure tap of the resistor is connected by tubing with an internal diameter of at least ¼ inch to an indicating pressure gauge which has very

low inertia.[4] The pressure gauge is housed in a suitable mounting provided with a scale derived from a calibration curve of the resistor. The combined unit is called a 'puffmeter.' It is designed for use as a screening device in physicians' offices and hospital wards.

The coefficient of correlation between data obtained with this device and the maximum breathing capacity in normal subjects and in patients with varying degrees of pulmonary disability was 0.89 (fig. 3). When a similar series of data was correlated with the ½-second vital capacity, the coefficient was 0.93.

Recording Devices. The other apparatus designed for use with this flow resistor is a polarized capacitance transducer unit which converts the pressure caused by the resistor into electrical energy recordable on a standard direct-writing electrocardiograph, e.g., a Sanborn Viso-cardiette. The circuit diagram is shown in figure 4. The capacitance transducer contains a stretched, 2-mil aluminum diaphragm with an effective diameter of 1¼ inch. It should be emphasized that this unit will reliably record a sustained signal for a period of not more than 0.3 second before exponential decay occurs. However, the peak flow rate is faithfully reproduced by this device and the response time is as fast as that of the electrocardiographic needle. The value of the calibrating condenser falls within the linear range of the flow resistor. The actual flow rate is calculated by comparing the amplitude of the deflection with that of the calibrating signal. The calibrating signal, produced by a capacitor, may be equated to a standard pressure imposed on the transducer and is most conveniently set at about 1 inch of water.

[4] Magnehelic gauge manufactured by F. W. Dwyer and Co., Chicago, Ill., no. 1040-F-1.

SUMMARY

The characteristics of a porous vitreous flow resistor for use in intermittent measurement of high flow rates are presented. Apparatus for indicating maximum expiratory flow rate and for making records of such phenomena on standard electrocardiographic equipment are briefly described.

The authors wish to acknowledge the suggestions and encouragement of Drs. Belding Scribner and Loren Carlson. We are grateful to Mr. Edmund Brand of the Electronics Section, Department of Physiology and Biophysics, and to Mr. Wayne Quinton of the Medical Instrument Shop, University of Washington School of Medicine, for technical assistance.

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A Simple Apparatus for Measurement of Pressure Volume Relationship in Respiration

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THE MECHANICS of breathing can be studied by means of the simultaneous registration of the breathing pressure and the tidal volume. Employing this method, Wirz (1) recorded the pleural pressure against the tidal volume in rabbits. The same principle has been used during artificial ventilation of animals (2, 3) as well as of man (4). The respiratory pressure volume diagram during spontaneous respiration has been recorded on the screen of a cathode-ray oscilloscope by DuBois and Ross (5) in dogs, and by Mead and Whittenberger (6) and McIlroy, Marshall and Christie (7) in man.

Buytendijk (8) introduced a practical technique for the study of the mechanics of human breathing, utilizing a comparison of esophageal pressure with lung volume. Although the intraesophageal pressure is slightly greater than the intrapleural pressure, its respiratory fluctuations are an exact reflection of the variations in the pleural pressure (9). The relationship of the tidal volume to the intraesophageal pressure in man has been established by the use of such electronic apparatus as the air-flow meter (6, 7).

The apparatus to be described differs from earlier comparable techniques employed in studies of human subjects in that no electrical transducers are employed and the apparatus used is relatively inexpensive.

APPARATUS

The tidal volume of the subject is obtained from a B.M.R. Krogh spirometer containing a mixture of air and oxygen. A fan circulates the gases, and soda lime in the spirometer absorbs the carbon dioxide produced. Pure oxygen is added intermittently to keep the oxygen concentration approximately constant. The oxygen consumption of the subject does not significantly affect the spirometer volume during one respiratory excursion. The spirometer is balanced at the distal edge of the bell by a long rubber string from the ceiling. The pressure difference between the outside and the inside of the spirometer at different settings of the bell is less than 0.1 cm water.

Received for publication August 4, 1955.

The intraesophageal pressure is recorded through a Porges neoplex duodenal tube having an external diameter of 2–4 mm and an oliveless tip. In accordance with the procedure of Fry and coworkers (9), one end of the tube is inserted into a cylindrical-shaped, thin-walled latex balloon measuring 12–15 cm in length and 11 mm in width. The opening of the balloon is securely tied around the tube so that the cavity of the balloon is air-tight. The lumen of the balloon communicates with the lumen of the tube through several holes in the tube wall.

The balloon containing the tube is inserted through the nose of the patient and passed to the lower part of the esophagus of the subject. The free end of the tube is connected to a torsion wire manometer[1] turning a small mirror around a vertical axis. The balloon is filled with approximately 4–7 ml of air so that it is neither emptied nor stretched by the intraesophageal pressures.

The mirror manometer and a small electric lamp are placed on the spirometer bell (fig. 1). The lamp emits a narrow beam of light which is reflected by the manometer mirror onto paper. When the room is darkened, the movement of a point of light can be observed on the paper during the patient's respiration; this can be traced with a pencil. If a permanent record is desired, the paper should be photosensitive and the room completely dark.

The time sequence is registered on the photographic records by means of a synchronous machine which closes the light current five times a second, so that the curve will be composed of small points appearing at intervals of 0.2 seconds.

A microswitch controlled by the position of the spirometer bell regulates the currents to a stepping relay. This relay closes the light current during one respiratory cycle and opens it during the next. Hence, exactly one respiration can be photographed.

The amplitude of the movement of the spirometer and of the manometer mirror changes less than 5% when constant volume variations of a frequency of 0–160/min. are applied to either the mouthpiece or the outside of the latex balloon.

[1] Generously made available by Dr. R. Elmquist, Elema, Hagalund, Sweden.

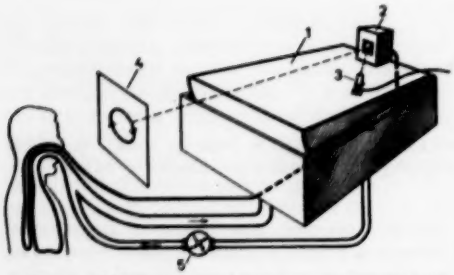

FIG. 1. Diagram of apparatus. *1*, spirometer bell; *2*, manometer (mirror revolves around vertical axis in response to intraesophageal pressure changes); *3*, light source; *4*, movements of light point on semitranslucent paper; *5*, fan.

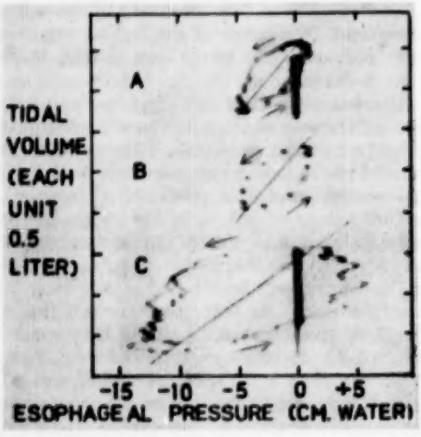

FIG. 2. Respiratory pressure volume relationship in seated patients, recorded on photosensitive paper. *A* and *B*, *R.A.*, male, aged 35, without pulmonary pathology. Respiratory frequency in *A* 19 and in *B* 25/min.; compliance 0.19 l/cm water; R in *A* 2.0 and in *B* 2.1 cm water/l/sec. calculated from areas and in *B* 2.0 calculated from inspiratory points. *C*, *S.L.*, female, aged 46, with asthmatic bronchitis. Respiratory frequency 14/min.; compliance 0.10 l/cm water; R 9.0 cm water/l/sec. calculated from curve area.

Within that frequency range there is no appreciable phase shift between the pressure and volume registrations if the manometer is sufficiently damped.

The magnitude of work required to move the spirometer is derived from measuring the pressure in the mouthpiece rather than that in the esophagus. The pressure variations then recorded are those needed to move the spirometer, while the area of the pressure volume curve represents the work required to move the spirometer. The spirometer work is determined after each record of the regular curve by turning the three-way stopcock so as to obtain the pressure in the mouthpiece.

The Curve. The point representing the pressure volume relationship travels on the photographic records to the left and downward during inspiration and shifts to the right and upwards during expiration (fig. 2).

The lung compliance is the slope of the line joining the highest and lowest points of the curve (2, 4, 6).

The work lost as a result of the nonelastic repiratory resistance is the area contained within the loop. The inspiratory nonelastic work is represented by the area on the inspiratory side of the compliance line and the expiratory work by the area on the expiratory side of that line (2, 4). A planimeter is used to measure the areas.

The respiratory resistance is given in the following equation, expressed as suggested by DuBois and Ross (5)

$$\frac{P}{V_D/t_D} = K_1 + K_2 \frac{V_D}{t_D} \qquad (1)$$

where with regard to the interrupted curves, V_D is the volume change from one point in the broken curve to another, t_D the time between the points, P the mean pressure difference between the compliance line and the two points, and K_1 and K_2 two constants. $\frac{P}{V_D/t_D}$ represents the resistance at a certain flow and is called R.

If the velocity pattern during inspiration is assumed to be a sine wave, the inspiratory resistance can also be described in the following relationship deduced by Otis, Fenn and Rahn (4)

$$W = \tfrac{1}{2}KV^2 + \tfrac{1}{4}K_1\pi^2 f V^2 + \tfrac{2}{3}K_2\pi^2 f^2 V^2 \qquad (2)$$

where W is the respiratory work during inspiration, $\frac{1}{2f}$ the time of inspiration, V the tidal volume, K the inverse of the compliance, and K_1 and K_2 the same constants as in *equation 1*. After subtracting the elastic work of inspiration, $\frac{1}{2}KV^2$, and rearranging the equation, R can be obtained from

$$\frac{8W_r t}{\pi^2 V^2} = K_1 + K_2 \frac{4V}{3t} = R \qquad (3)$$

where W_r is the inspiratory work due to the nonelastic resistance and t the time of inspiration.

From the work, time and volume displacement values obtained from the curves, R can be easily calculated. *Equation 3* can be applied to inspira-

tion or expiration if their velocity curves have a sine wave contour and to the whole respiratory cycle if its velocity pattern is one sine wave. In the latter case time marking can be dispensed with, if the respiratory frequency is counted.

The heart beat will on occasion affect the intraesophageal pressure record. The impact of the cardiac action will in such cases cause an error in the calculation of R from the points of the broken curve. That error is minimized by analyzing a number of curves and not using points relatively close to the compliance line. When the R value is derived from the area values, the cardiac distortion of the curve seems to be of less importance.

The R value obtained from the points usually agrees with the value calculated from the area with *equation 3*. The breathing pattern is probably in most cases sufficiently like a sine wave to permit the determination of R from the areas.

Curves from a subject with normal lungs and a patient with asthmatic bronchitis are shown in figure 2.

The apparatus and the procedure described are currently employed in this laboratory to study the mechanics of breathing in patients with various pulmonary conditions.

SUMMARY

An apparatus is described which utilizes non-electrical transmissions for the determination in man of intraesophageal pressure and tidal volume relationships. Lung compliance, work of breathing and respiratory resistance are easily calculated from the curves obtained.

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A Sensitive Electrical Weighing Device for Human Subjects[1]

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THIS REPORT describes an electrical weighing device designed to meet the requirements for convenient, precise, continuous weighing of human subjects, as in thermal stress experiments, where weight loss may range from 0.5 to 70.0 gm/min. (1). Mechanical scales used up to now for this purpose by Lombard (2), Adolph (3), Krogh and Trolle (4), Nielsen (5), and others, and other scales available commercially[2] are slow, require painstaking care in use, do not provide a truly continuous indication and do not permit remote recording.

The electrical weighing device has a capacity of 100 kg and an ultimate sensitivity of more than 50 mg. Its working sensitivity of 1 gm/min. is at present limited by instability of the particular amplifier used. When carrying an 80-kg man in an adjustable chair-couch, the device has a natural frequency of 2.5 cps; cardiac and respiratory ballistic movements may be measured or may be damped out electrically for mean weight loss determinations. The weighing pickup or transducer may be used remotely at distances up to 50 feet from the amplifier and continuous recording apparatus. The weighing device is adaptable to the multichannel instrument array required for data collection in thermal stress studies.

The weighing transducer is the heart of this weighing device. Its operating principle is illustrated in figure 1. When the weight of a subject is suspended from the central shaft, *13*, force is transmitted to the spring support, *7*, causing compression of the special helical spring, *10*. Movement of the spring support and the shaft are proportional to the weight. The shaft carries a magnetic core, *11*, whose movement is detected electrically by the differential transformer, *12*, inside the spring. Since the electrical output is proportional to displacement of the core, the output of the whole transducer is proportional to weight. The shaft, transformer and spring are concentrically mounted and are not in contact. Friction of the shaft in its supports is minimized by use of ball bushings. The transducer is shown in complete assembly in figure 2.

The novel components of the transducer deserve brief mention. The helical compression spring is made of a new spring alloy, Iso-elastic,[3] having very low hysteresis, high linearity of load vs. deflection, low drift and creep; and very low thermal coefficients of elastic moduli and of linear expansion. The ball bushing[4] provides extremely low kinetic friction and precise alignment for the moving shaft, employing rolling balls for sliding motion in a manner analogous to their use for rotary motion in an ordinary radial ball bearing. The differential transformer[5] possesses many superior properties (6) which entitle it to a wider application in physiological research. As a displacement detector it has a very long linear range; high linearity of electrical output vs. displacement, high full scale electrical output; high sensitivity, coupled with infinite resolution; and low impedance input and output, which results in low noise and easy cable matching for remote pickup. It is rugged and is easily mounted and dismounted for reuse.

The total weighing device includes the weighing transducer and other units shown in figure 3. The transducer itself is placed at the end of the long shielded cable, separating it from the other units which are grouped together. The weighing procedure involves both null-balance and deflection techniques. To cancel out initial weight, the micrometer screw in the balance unit is turned, to shift the position of the magnetic core in a fixed differential transformer, until this coincides with the position of the core in the weighing transducer. When core positions correspond, no net output voltage is obtained from the pair of differential transformers, which are shown connected in series

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Received for publication September 30, 1955.

[1] This investigation was supported by a contract AF 18(600)-96 between the United States Air Force, Wright Air Development Center, Wright-Patterson Air Force Base, Ohio, and St. Louis University.

[2] August Sauter KG, Ebingen, Western Germany, Catalog No. 25 on balances (U.S. Office, 101 Park Ave., New York City). Buffalo Scale Company, Buffalo, N. Y.

[3] John Chatillon and Sons, 85 Cliff St., New York City.

[4] Thomson Industries, Manhasset, N. Y.

[5] Schaevitz Engineering Co., Camden, N. J.

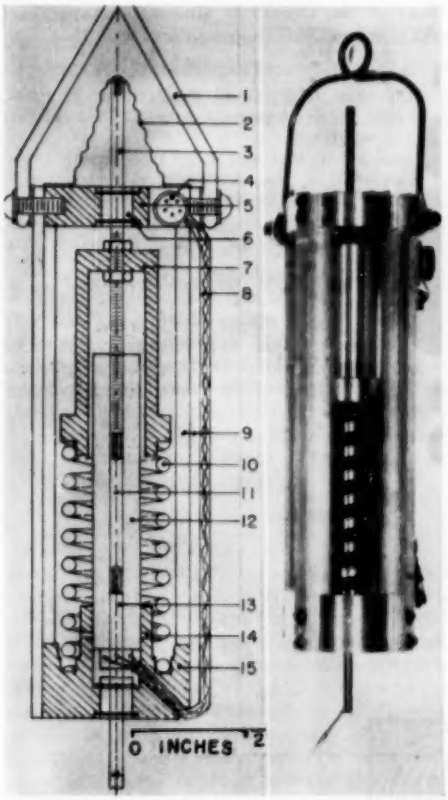

FIG. 1. (*left*). Weighing transducer: *1*, stainless steel suspension loop; *2*, plastic film dust cover; *3* and *13*, shaft, nonmagnetic stainless steel, commercial ground rod; *4*, electrical receptacle; *5*, upper support plate, Dural; *6*, ball bushing, Thomson A-4812; *7*, upper spring support, Dural; *8*, electrical output cable; *9*, support plate tie beams, Tee section, Dural; *10*, helical compression spring, Chatillon 'Isoelastic'; *11*, magnetic core, relay steel; *12*, differential transformer, Schaevitz 1000-SL; *14*, set screws, Allen head type, for zero set of transformer; *15*, lower support plate, Dural.

FIG. 2 (*right*). Weighing transducer, completely assembled.

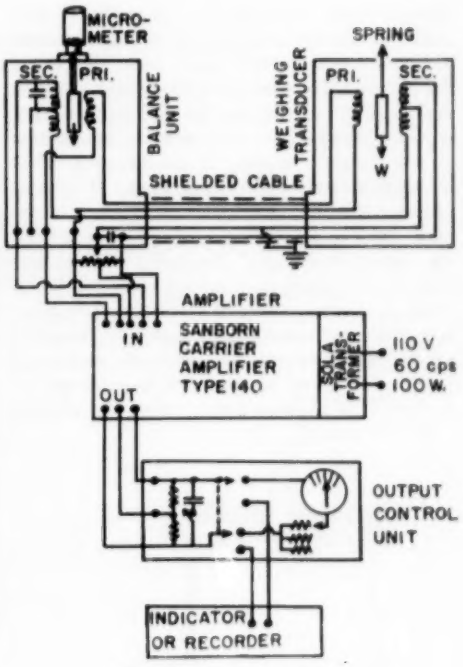

FIG. 3. Total weighing device.

opposition. Changes in weight will result in a net voltage appearing at the amplifier input terminals. Dynamic weight variations result in a high-level electrical signal appearing at the amplifier output then entering the output control unit, where connection may be made to a self-contained galvanometer, or external indicator or recorder.

The amplifier used in this first model is a carrier type,[5] containing a 3-volt, 2-kcps source of excitation for the differential transformers, a multistage tuned high-gain amplifier strip, and phase-sensitive detector or demodulator. While this amplifier has demonstrated admirable performance in many diverse physiological applications; improved sensitivity and stability of the weighing device will be attained when the present amplifier is replaced by a newer model having much greater stability, higher sensitivity and higher linearity.

The device may be usefully applied to many problems in physiological research, including: *1*) determination of the total weight loss in thermal stress. After correction for dripping sweat, expired water vapor and O_2–CO_2 exchange, evaporative heat loss may be calculated for correlation with calorimetric determinations; *2*) comparison of weight loss due to sweating, with regional sweating rate determinations and further validation of regional 'weighting' factors, such as are used by Weiner (7) and by Hertzman *et al.* (1) for calculation of total sweating rates; *3*) observation of the time course of total sweating rate, for detection of any cyclic sweating of widespread synchronous

[5] Sanborn Model 140, Sanborn Co., 39 Osborn St., Cambridge, Mass.

nature, which could result in intermittent heat exchange through this channel of heat transfer. Regional cyclic bursts have been shown by Randall and Hertzman (8) with capsule methods, and by Albert and Palmes (9) with infrared techniques. Observations may provide information on the dynamic response of the sweating servomechanism and its neural components; *4*) ballistocardiography, for observation of changes in the mean value and nature of cardiac output, including detection of any developing beat-to-beat irregularities, which may indicate early circulatory strain in thermal stress; *5*) specific gravity determinations, and estimation of lean body mass; *6*) general application to any automatic continuous weighing problem; *7*) precision measurement of any static or dynamic force, within the working range of the device; in studies of acceleration effects on survival equipment and so forth.

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Oxygenation of Circulating Blood by Dispersion, Coalescence and Surface Tension Separation

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TO PRODUCE the large interface between erythrocytes and oxygen needed to achieve promptly full oxygenation of large volumes of venous blood, a thin film of blood on a large area can be exposed to oxygen (1) or oxygen can be bubbled into blood (2). The rapid advance in cardiac surgery during the last two decades has led to great improvements of these two methods and has divided contemporary investigators into 'blood filmers' and 'blood bubblers.' The instrument to be described is based on the principle of dispersing fine oxygen bubbles into blood (3).

APPARATUS AND METHODS

Venous blood from the caval system of heparinized dogs is withdrawn by a finger pump[1] and circulated through a lucite cylinder[2] of 3-inch diameter and 7-inch height (fig. 1). Venous blood enters the lower compartment at an oblique angle to make it swirl. Through a siliconed porcelain disc of 4.4 μ pore size,[3] oxygen is forced into the blood with a pressure of about 8–10 lb. Siliconing of the microporous disc produces very large bubbles in water but medium-sized bubbles in blood. The large interface between the oxygen bubbles and the blood is augmented by the created turbulence and makes prompt and full oxygen saturation with medium-sized bubbles in a small volume possible. These bubbles, however, are not large and buoyant enough to rise against the current to the surface and would cause oxygen embolism. Therefore, they are made to coalesce into large bubbles by bringing them into contact with the Antifoam A compound.[4] In order to accomplish this, another large surface is created by a tight roll of fiberglass screen[5] which is coated with the ether-soluble, nontoxic silicone compound. The large oxygen bubbles which emerge from the roll of fiberglass screen are collected and directed away from the arterial outlet by a triangular piece of fiberglass screen whose base is fastened with chloroform to the lucite cylinder. The mesh size of this screen is so small that the large elastic gas bubbles cannot pass through, whereas the oxygenated and defoamed blood filters via the arterial outlet into the left subsubclavian artery.

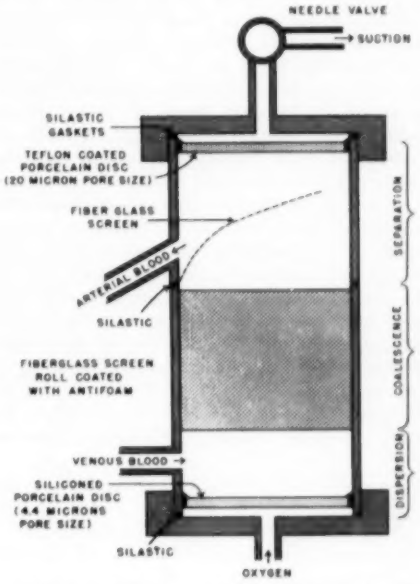

FIG. 1. Diagram of blood oxygenator based on changes of surface tension in gas dispersion, coalescence and separation.

After the separation of the excess oxygen and carbon dioxide from the circulating blood the large gas bubbles collect underneath the upper microporous porcelain disc. This disc of 20 μ pore size is made hydrophobic by coating it with Teflon[3] and therefore blood is repelled from the nonwettable surface, whereas gas can pass through

Received for publication October 13, 1955.

[1] Model T-6 Sigmamotor pump, Sigmamotor, Inc., Middleport, N. Y.

[2] Lucite Acrylic resin tube, E. I. DuPont de Nemours, Chicago, Ill.

[3] Generous supply and invaluable information on properties of porcelain filter discs by George V. Jordan, Jr., Selas Corp. of America, Philadelphia, Pa., is gratefully acknowledged.

[4] Dow Corning Corp., Midland, Mich.

[5] Sears, Roebuck and Co., Catalogue No. 99 J 06874.

it. Rapid removal of free gas is accomplished by a small amount of suction which is supplied from another opening of the same finger pump and which can be regulated by a plastic needle valve.[6] This principle of surface tension separation of free gas from an aqueous phase by means of a hydrophobic porous membrane (4) makes oxygenation of 2 liters of blood per minute in a simpler and smaller closed system possible.

The system is made airtight by gaskets made of 35% silastic suspension in xylene[6] and it is held together by stainless steel rods and thumbscrews. The volume of the chemically sterilized cylinder and tubings amounts to about 450 ml, and because of the low cost of the commercially available materials disposable units can be produced.

RESULTS

With this instrument the same blood flow and oxygenation can be produced as with a previously described blood oxygenator (5). However, the newly employed principle of surface tension separation of free gas from the liquid phase simplifies and alters the procedure to such an extent that new physiological effects can be achieved. The flow of blood is almost nonpulsatile which is due to the frequent and gradual compressions of a rubber tube by the finger pump, as well as the dampening effect of the large gas bubbles after coalescence. This continuous flow of blood into the aorta prevents the previously observed overloading of the coronary circulation during hypothermia below 20°C (6) and thus produces an almost bloodless operating field in open intracardiac surgery. Therefore, instead of the cumbersome and traumatic procedure of double cannulation of the cerebral and systemic circulation to reduce excessive coronary flow, a single catheter in the left subclavian artery suffices to perfuse all vital organs with the proper amount of oxygenated blood.

The reduced volume and the shortened circuit makes the priming with blood unnecessary since the instrument can be filled with Ringer's solution. This eliminates the hazards of incompatible blood and viral infection and reduces hemolysis. The dose of heparin in hypothermic animals can be lowered to 1.5/kg and thus hemostasis after thoracotomy can be accomplished by transfusions of decalcified blood alone.

The elimination of valves, bubble traps, leveling devices and electronic feedback circuits reduces the chances of technical errors which have marred successful application of most large, complicated and expensive instruments in the past.

[6] Palo Laboratory Supplies, Inc., New York City.

SUMMARY

By the utilization of the surface-active properties of microporous membranes and screens a small enclosed chamber can serve as an efficient blood oxygenator.

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Delay in Transmission of a Pressure Impulse Through a Cardiac Catheter and Vinyl Plastic Tubing

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THE TRANSMISSION time of a pressure impulse through a cardiac catheter using a Hamilton manometer and recording system was found to be 0.01 seconds by Cournand *et al.* in 1946 (1). This value was subsequently employed by Coblentz *et al.* (2) in the determination of the temporal relationships between electrical and mechanical events within the right side of the heart.

In the course of recent investigations of the timing of events on the left side of the human heart (3) it became apparent that the transmission time as measured by our electronic equipment might be different, and that it would be desirable to repeat these studies. Furthermore, our recording system permitted measurements more precise than heretofore possible. We studied the transmission time of the pressure impulse not only through a cardiac catheter of standard length (100 cm), but also through a 122 cm length of thick-walled vinyl plastic tubing[3] such as we employ for direct measurements by means of a needle thrust through the wall of the human heart at operation (4).

METHODS

A sudden pressure impulse was generated by smartly tapping the end of a plunger of a 50 cc syringe which was partially filled with water. A round electrocardiographic electrode was cemented to the end of the plunger, and one electrocardiographic lead wire was soldered to it. The other wire of the lead was attached to the hammer with which the plunger was tapped, causing an electrical impulse to be generated simultaneously with the mechanical impulse.

The vinyl plastic tubing was 122 cm long, with a bore of 1.9 mm and outside diameter of 6.6 mm. It was provided by the manufacturer with Luer adaptors, one of which was attached directly to the syringe tip, the other to the stopcock leading into a P23A Statham pressure transducer. When the delay in transmission through the catheter was tested, the distal end was fixed securely to the syringe tip by means of a Tuohy-Borst adaptor.

Two channels of a four-channel single-gun photographic oscilloscopic recorder[4] were used for the actual measurements. In this instrument the beam is split into four traces by an electronic switch. At slow speeds each trace appears as a solid line, but at more rapid speeds the trace becomes an interrupted one. In order to take advantage of the electron switching rate of the oscilloscope for timing it was necessary to generate a paper speed far in excess of that possible with conventional paper-drive mechanisms. This was accomplished by pulling the photographic paper manually in a darkened room. After some practice, it was possible to attain a paper speed of approximately 1750 mm/sec., sufficient to separate the oscilloscopic traces of the two channels, representing the electrical and mechanical events, into their component dots.

The electron switching rate of the oscilloscope was known to be 0.00133 seconds. This rate was originally set by an audio-oscillator and found by checking with 60-cycle alternating current to be accurate to better than 1%. It was only necessary to count the dots between the inscription of the electrical and pressure impulses and multiply by the above figure to obtain the desired latent period (fig. 1).

On both the electrocardiographic and pressure channels the sensitivity was made high enough to cause the trace to take off abruptly. It was found that the height of the initial fluid pressure in the syringe had no detectable influence on the transmission time, and this factor was therefore not controlled.

In the first series of experiments the syringe was supported in an upright position by a rubber clamp. It was observed that the initial evidence of pressure transmission was a brief negative deflection, of small amplitude. This was then followed by a larger sustained pressure rise. The reason for this initial negative deflection was not

Received for publication September 28, 1955.

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[3] Both catheter and tubing are made by U.S. Catheter and Instrument Co., Glens Falls, N. Y.

[4] Made by Electronics for Medicine, Inc., White Plains, N. Y.

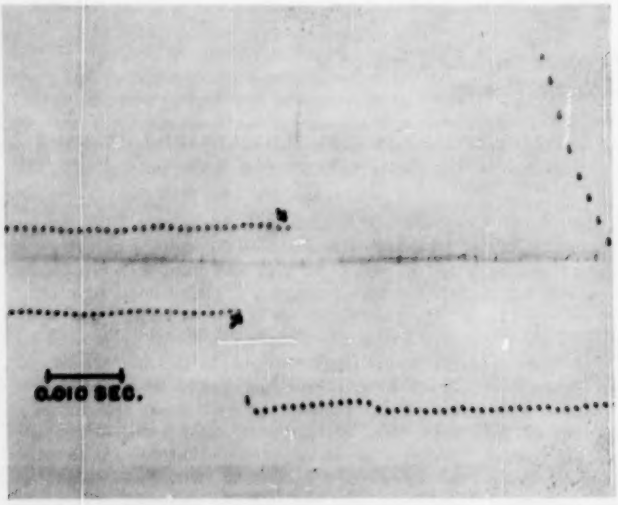

FIG. 1. One of trials performed with standard cardiac catheter. *Top trace*: pressure wave; *bottom*: electrical impulse. The delay amounted to six dots. This figure, multiplied by electron switching rate (0.00133), gave a delay of 0.0079 sec.

clear. It may have represented the passage of an expansile impulse along the walls of the tubing, initiated by jarring of the system. The velocity of this impulse was presumably greater than that of the pressure wave transmitted through the fluid, probably because of the greater density of the tubing.

In order to test this hypothesis, the tip of a catheter was occluded by a tightly fitting wire and the experiment repeated. Tapping the syringe plunger now produced a rapid negative deflection which was not followed by the large positive wave encountered with a patent catheter, and confirmed that the negative deflection was not related to pressure transmission through the fluid system.

In order to eliminate this factor as much as possible, the shoulder of the syringe was supported on a rigid surface to prevent the jarring impulse from being transmitted to the walls of the tubing. With this system, the first impulse to be recorded was a positive one, which presumably represented transmission of the wave front through the fluid.

RESULTS

Three sets of experiments were carried out. We first tested a no. 7F cardiac catheter 100 cm long, with an outside diameter of 2.3 mm and a single terminal opening. In 12 trials the delay between the electrical and pressure impulse measured 0.0069 ± 0.0006 seconds.

In a total of 7 trials with the vinyl plastic tubing 122 cm long, the transmission delay measured 0.0059 ± 0.0007 seconds. It is of interest to compare these figures with the transmission time of sound through water. Using the figure of 4700 ft/sec. as the speed of sound in water, it would take 0.00085 seconds for a sound wave to traverse an equal distance in water.

Finally, the syringe was attached directly to the transducer by means of a stopcock and the experiment repeated. In a total of 12 trials the delay measured 0.0013 seconds.

SUMMARY

Determination was made of the delay in transmission of a pressure pulse through a cardiac catheter 100 cm long and vinyl plastic tubing 122 cm long. The system employed a Statham pressure transducer and cathode-ray oscilloscopic recorder. The delay through the catheter was found to be 0.0069 seconds, and through the plastic tubing, 0.0059 seconds.

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Direct Measurements of Aortic Blood Pressure in Unanesthetized Rats[1]

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BLOOD pressure in small animals has been measured by direct and indirect methods. In direct methods, the hemodynamics of the experimental subjects (1) are greatly altered by anesthesia and surgical procedures and the blood pressure observed under such conditions deviates by a variable amount from the true normal level. Moreover, in small animals, repeated direct observations are often impossible because the techniques involve the destruction of vessels or of the entire animal. In indirect methods based on the detection of arterial inflow distal to a slowly deflating cuff on the foot, tail or ear of the animals, anesthetics and surgical procedures may be avoided. However, these methods frequently manifest several defects, e.g. imprecision of the end point (2), tendency towards subjective errors (2), lack of correlation between the readings and those obtained with direct methods (3, 4) and discrepancy between the readings in repeated observations due to edema as a result of tissue damage in the part under observation (4). The technique described in the present report is free from most of these defects and permits a continuous direct record of aortic blood pressure (through an implanted tube) in unanesthetized rats.

METHODS AND MATERIALS

Rats were prepared in a manner similar to that described by Still and Whitcomb (5, 6), The abdomen of a rat was opened under ether anesthesia and a no. 10 polyethylene tube was introduced into the inferior aorta in a cephalic direction for a distance of 1.5–2 cm. The other end of the tube was carried out of the abdomen and beneath the skin to the dorsal aspect of the neck where it was exteriorized. The tube was filled with normal saline and sealed. The incision was closed and the animal was allowed to recover for at least 5 days before it was used experimentally.

Blood pressure in these animals was recorded by the direct writing pen of a Brush oscillograph attached to a Brush universal analyzer[2] connected with a Statham physiological pressure transducer (Model P 23 D).[3] The details of this technique of measurement of blood pressure are given in a separate communication (7). In the present study, the transducer was suspended over the rat to provide the latter some latitude of movement, and a syringe was connected to the side arm of the transducer for injection of heparin or other drugs intra-arterially (fig. 1).

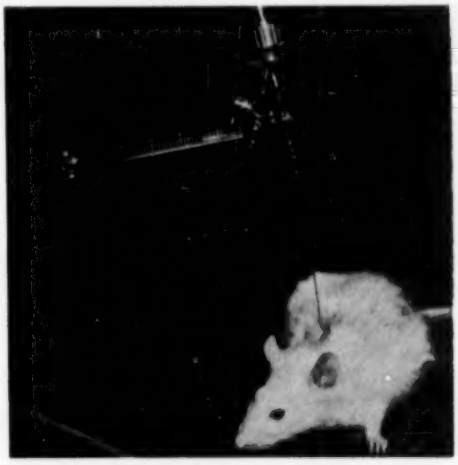

FIG. 1. Unanesthetized rat prepared for measurement of blood pressure. Pressure transducer, suspended from above, is connected to polyethylene tube inserted into abdominal aorta of the rat.

The rat was gently held in restraint by gloved hands to limit its free movement. The plug was then removed from the exposed end of the tube. When the tube was not blocked, blood flowed freely from it. When blocked a 27-gauge needle was inserted into the tube and a small amount (0.1–0.2 cc) of saline containing heparin (0.01%) was slowly forced through it from a syringe. When

Received for publication February 28, 1955.

[1] This work was supported in part by a grant (H 1739) from the Public Health Service and in part by a grant from the Washington Heart Association.

[2] Brush Development Company, Cleveland, Ohio.

[3] Statham Laboratories, Beverly Hills, Calif.

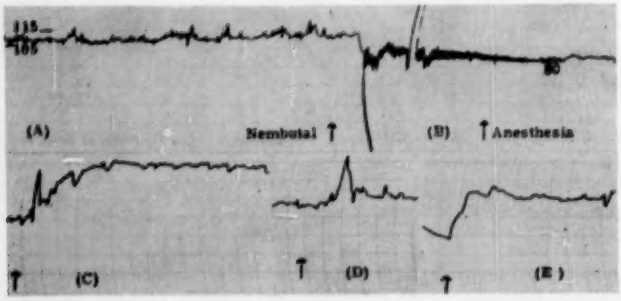

FIG. 2. (*A*) normal blood pressure of an unanesthetized rat (200 gm) measured directly from abdominal aorta; (*B*) hypotensive effect of Nembutal (9 mg) given intra-arterially in same rat; (*C*) pressor effect of *l*-arterenol (0.2 mg) injected intraperitoneally in another rat (225 gm); (*D*) and (*E*) effects of putting the tail in hot water and pinching the skin, respectively, in the second rat.

the blood flowed freely from the tube, about 0.2 cc of 1% heparin solution was injected intra-arterially. The needle was then disconnected from the syringe and fitted to the transducer.

RESULTS

Blood pressure at any time during the experiment was determined from the tracing of the oscillograph on comparison with the calibration curve made at the beginning of the experiment. Mean blood pressures in the four rats recorded so far were 70, 90, 95 and 110 mm Hg. Figure 2*A* shows the blood pressure tracing in one such rat. The pressures are slightly lower than actual, because of the length of tube connecting the aorta to the transducer and because the transducer was a few centimeters above the aorta. In another rat the blood pressure went up when its tail was put in hot water (fig. 2*D*) and when its skin was pinched (fig. 2*E*). A rise was also noticed when the rat was excited or in motion. The depressive effect of anesthesia (Nembutal) was observed even before the animal was fully anesthetized (fig. 2*B*). Blood pressure in these rats responded promptly to the usual pressor and depressor agents like *l*-arterenol (fig. 2*D*), Pitressin, histamine and so forth.

DISCUSSION

Marquardt and Hildebrand (8) described a technique for measuring blood pressure in the unanesthetized cat on 'exteriorization' of the femoral artery. As far as the smaller laboratory animals are concerned, the only analogous work that was found is the report of Poel (9). Poel 'exteriorized' the carotid artery of the rat and recorded blood pressure up to 36 hours;[4] however, the rats did not survive for extended periods.

[4] Personal communication.

The method described in this paper provides a means of recording direct blood pressure in unanesthetized rats for periods up to at least a month. At the time of preparing this paper rats have been maintained in apparent good health for as long as 30 days with their implanted tubes still patent. It remains for future work to determine how long beyond this period such rats survive and what variations in blood pressure, if any, will be obtained on repeated measurement.

SUMMARY

A technique for continuous recording and measurement of blood pressure in unanesthetized rats directly from the abdominal aorta (through an implanted tube) has been described. Mean aortic blood pressures of four rats so far recorded were 70, 90, 95 and 110 mm Hg. Blood pressure thus recorded responded promptly to physiological stimuli and drug action.

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